SUPPLEMENT TO AMERICAN JOURNAL OF PUBLIC HEALTH
VOL. 26, NO. 3, MARCH, 1936

Sixth Annual Year Book

AMERICAN PUBLIC HEALTH ASSOCIATION

1935-1936

AMERICAN PUBLIC HEALTH ASSOCIATION
50 WEST 50TH STREET, NEW YORK, N. Y.

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AMERICAN PUBLIC HEALTH ASSOCIATION

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Foreword to Sixth Annual Year Book

WHEN the first *Year Book* of the American Public Health Association was presented to the membership in 1931, it was a device calculated to accomplish two purposes; first, prompt publication of scientific committee reports read at the Annual Meeting under one cover rather than in scattered installments throughout the year; and second, to release the two hundred odd pages of the *Journal* previously given over to committee reports for the admission of timely contributed articles having no Annual Meeting origin.

A considerable volume of material representing cumulative and current information relating to the Association which the *Journal* had carried heretofore seemed more appropriate for publication in the *Year Book*, and was accordingly included—such things as the Constitution and By-laws, lists of committees, and section councils, the report of the Executive Board to the Governing Council, etc.

This pattern was followed without alteration for 5 years, while a picture was forming in the minds of the Editorial Committee of what the *Year Book* could be in order to render its best service to the membership. There finally evolved a conception of the *Year Book* as a depository of the things mentioned above and also of certain information significant to health workers and classified as—unobtainable elsewhere, accessible elsewhere from sources not generally familiar, or perhaps easily available but worth repeating for constant and ready reference. Obviously, to include all would be to attempt to bind the Surgeon-General's Library in one volume. We can begin modestly, however, to add to the *Year Book's* usefulness, and this year it is presented with several new sections.

The lists of state, county, and city health officers are included, with indications of American Public Health Association membership affiliations. Other innovations are: a list of universities in the United States and Canada conferring public health degrees, together with brief statements of entrance requirements; a list of American foundations making grants of amounts in excess of $5,000 for medical and public health purposes in 1934; a list of cities surveyed by the American Public Health Association; lists of winning and honorable mention cities and counties in the Rural and City Health Conservation Contests.

The only omission over previous years is the report of the Executive Board to the Governing Council. This is a lengthy mimeographed document which has been distributed in full to the members of the Council. It covers in detail the work of the administrative office and of the five standing committees. Its permanent value outside the Association's archives is questioned, and it is, therefore, omitted from the 1935–1936 *Year Book*. Copies are available in the headquarters office and will be sent upon request to anyone interested.

Suggestions from the membership are invited for future *Year Book* improvements. They will be considered for inclusion in later volumes providing they have value for a fair segment of the total membership.

The American Public Health Association

Its Objectives and a Pledge of their Attainment

OUR permanent objective is the effective control of preventable disease, and the security of health for all the people.

The first necessity to attain this objective is:

A full-time health service with trained personnel for every community, and provision of adequate public funds for its support.

The first fruits of such organization and support of public health knowledge and resources will be:

1. Reduction of the maternal mortality rates, so that the United States and Canada will be second to no nation in the safety of motherhood.
2. Securing normal growth of body and mind for children, and their training in the laws and personal practices of a healthy life.
3. Protection of life and limb and promotion of health for the working man and woman.
4. An adequate supply of safe milk for every community.
5. An adequate supply of pure water for every community.
6. Elimination of tuberculosis, malaria, hookworm disease, typhoid fever, diphtheria and smallpox, among the communicable diseases of which we have sufficient knowledge.

The American Public Health Association pledges to the health workers of the country and to the people of our member nations vigorous coöperation in carrying these projects to a successful conclusion.

CONSTITUTION AND BY-LAWS
AMERICAN PUBLIC HEALTH ASSOCIATION

As Adopted at the Fifty-eighth Annual Meeting, and Amended at the Sixty-fourth Annual Meeting

CONSTITUTION

ARTICLE I NAME

The name of this Association, incorporated under the laws of Massachusetts, is the American Public Health Association.

ARTICLE II OBJECT

The object of this Association is to protect and promote public and personal health.

ARTICLE III GOVERNING COUNCIL

A. *Composition:* There shall be a Governing Council consisting of:

1. The officers of the Association.

2. Thirty members of the Council, to be elected by and from the Fellowship of the Association, for three-year terms, one-third retiring each year. These members of the Council shall be nominated and elected as provided for in the By-laws.

If one of these members is elected a Section Chairman, Vice-Chairman, or Secretary, or appointed the representative of an Affiliated Society, a new Councilor to fill such vacancy shall be elected by the Governing Council.

3. The Chairman, Vice-Chairman, and Secretary of each Section.

4. Representatives to be appointed by Affiliated Societies as provided for in the By-laws. Such representatives shall be Fellows of the American Public Health Association.

5. The elective members of the Council of the Health Officers Section.

6. A representative to be designated by each regional branch. Such representative shall be a Fellow of the American Public Health Association.

B. *Terms:* Terms of Councilors shall begin at the end of the annual meeting when elected, and shall terminate at the end of the annual meeting at expiration of term; provided that Councilors shall have the right to attend meetings of the Council in an advisory capacity as soon as elected.

C. *Reëlection:* After two consecutive terms, an elective Councilor shall be ineligible for reelection to the Council during one Association year.

D. *The Officers of the Association* shall be the officers of the Council.

E. *Functions:* The functions of the Council shall be:

1. To establish policies for the Association and for the guidance of the Executive Board and the officers.

2. To establish Sections of the Association; to combine or discontinue them when necessary; to maintain coördination among them; and to formulate general rules governing the policies of the Sections.

3. To submit to the vote of the Association all resolutions which have received the approval of the Governing Council.

4. To elect and establish qualifications for Affiliated Societies, Fellows, and Honorary Fellows as provided in the By-laws.

5. To elect the Executive Board and the officers of the Association.

6. To receive from the Executive Board at its first session, at the time and place of the annual meeting of the Association, a definitely formulated statement of a program of the major activities proposed for the ensuing year. To determine at the annual meeting of the Association in general outline the allocation of Association moneys in the budget for the ensuing year. To require a report from the Chairman of the Executive Board in which the work, the accomplishments and the financial status of the Association during the year preceding such annual meeting shall be reviewed.

7. To publish after each of its meetings an abstract of the minutes of such meetings.

F. *A Quorum* of the Council shall consist of ten Councilors.

G. *Meetings* of the Council shall be called by the Executive Secretary at the request of the President, or at the request in writing of any twelve Councilors. In the latter case, the call to meeting, issued at least twenty days in advance, shall state the purpose of the meeting.

ARTICLE IV OFFICERS

The officers of this Association shall be a President, a President-elect, three Vice-Presidents, an Executive Secretary, a Treasurer, and the Chairman of the Executive Board. The officers, with the exception of the Chairman of the Executive Board and the Executive Secre-

tary, shall be elected by written ballot of the Governing Council as provided in this article and in the By-laws. The President-elect shall serve as such from the close of the annual meeting at which he was elected to the close of the next annual meeting, when he shall automatically become President. As President he shall serve to the close of the next succeeding annual meeting. Other officers shall serve from the close of the annual meeting when elected, until the close of the next annual meeting, and all officers shall serve in any case until their successors are elected and qualified. A majority vote of the Councilors voting shall be required to elect, and if no candidate receives a majority vote on the first ballot, the candidate receiving the smallest number of votes shall be dropped after each ballot in succession until a majority vote is obtained. The Chairman of the Executive Board and the Executive Secretary shall be elected by the Executive Board, which Board shall define the duties and authority of these officers, respectively.

ARTICLE V AMENDMENTS

This Constitution may be amended by a two-thirds vote of the Fellows of the Association present and voting at an annual meeting, provided that the specific amendment to be acted upon is published in the official publication of the Association not less than thirty days prior to the meeting, and provided further that the amendment has received the approval of the Governing Council.

BY-LAWS

ARTICLE I MEMBERSHIP AND DUES

A. There shall be seven classes of constituents. Of these, Fellows may be elected only from the United States, Canada, Mexico and Cuba. The other six classes of constituents may be elected from any country. The respective appellations, qualifications for election, and dues shall be as follows.

1. *Fellows:* Only professional health workers, from the United States and its possessions, Canada, Mexico and Cuba, who have been Members of the Association for at least two years, and of established professional standing (whether employed by public or private agencies or in independent private practice), shall be eligible for election as Fellows, provided that a Member shall be not less than thirty years old at the time the application for Fellowship is made, and provided, further, that the following persons shall be considered to have an established standing in the profession of public health, namely:

a. A person who has attained the degree of Doctor of Public Health, Doctor of Science in Public Health, Doctor of Philosophy in Public Health, or other equivalent degree, according to standards approved by the Executive Board of the American Public Health Association.

b. A person who has attained an academic or professional degree involving training in public health, and who has been regularly engaged in public health work for four years, having rendered meritorious service in the public health profession, either as a health officer or in responsible charge of work in a state or municipal department of health or other official public health organization.

c. A person who has done notable original work in public health or preventive medicine of a character to give him a recognized standing equivalent to that required for Fellows under paragraphs "a" and "b."

d. A person regularly engaged in public health work for at least five years, who has given evidence of special proficiency in the service of an official or unofficial public health organization, and who has attained a professional standing equivalent to that required for Fellows under paragraphs "a" and "b."

e. A teacher of public health or one of its constituent sciences. As such he shall have attained distinction as an expounder of the principles of public health or its constituent sciences and he shall have had at least five years' experience as a teacher of public health subjects. Any years of experience as defined in paragraphs "b" and "d" that the applicant may have had shall be considered the equivalent of the same number of years experience as a "teacher."

f. A person not covered by the above, who has made substantial contributions to public health work in his chosen branch of public health service, and who has attained a professional standing equivalent to that required for Fellows under paragraphs "a" and "b."

The application for Fellowship shall be made on an approved form and shall be sponsored by two Fellows of the Association who shall be Fellows of the Section with which affiliation is desired, provided, however, that when affiliation with a Section is not desired, the sponsors may be any two Fellows in good standing in the Association. Fellows without Section affiliation shall be known as unaffiliated Fellows.

When the application has been duly sponsored and otherwise completed, it shall be transmitted to the Administrative Office of the Association, which shall make note thereon of such knowledge as it may have concerning the standing of the applicant in the Association. The application shall be forwarded by the Administrative Office to the Secretary of the Section in which affiliation is desired, for the approval of the Section Council, and when acted upon by the Section Council, it shall be returned to the Administrative Office by the Secretary of the Section, after he has made endorsement on the application of the action of the Section Council. When the application is for unaffiliated Fellowship, the Executive Board of the Association shall act in place of the Section Council. When the application has been approved by a majority of the Section Council or the Executive Board, as above provided, it shall be voted upon by the Governing Council, provided the name of the applicant shall have been officially published at least fifteen days in advance, and provided further that the application shall have been approved by the Committee on Fellowship and Membership.

A Fellow may belong to and vote in only one Section, but such affiliation may be transferred to another Section if approved by vote of a majority of the Council of the latter Section. Unaffiliated Fellows may become affiliated with a Section if approved by vote of a majority of the Council of the Section with which affiliation is desired.

The right to hold office or to serve as chairman of a committee in the Association shall be limited to the Fellows of the Association, whether Section Fellows or unaffiliated Fellows. The right to hold office or to serve as chairman of a committee in a Section shall, however, be limited to the respective Fellows in such Section. This provision shall not prevent the election of a Vice-President of the Association who may not be a Fellow.

The dues of Fellows shall be $10.00 per year.

2. *Honorary Fellows:* Honorary Fellows may be elected by the Governing Council for distinguished service in public health. Honorary Fellowship shall not include voting power or payment of dues.

3. *Members:* Persons professionally engaged or interested in public health work shall be eligible for election as Members when sponsored by two Fellows of the Association. They may serve on committees, except as chairmen. Dues $5.00 per year. A Member may belong to only one Section, but such affiliation may be transferred to another Section if approved by vote of a majority of the Council of the latter Section. Unaffiliated Members may become affiliated with a Section if approved by vote of a majority of the Council of the Section with which affiliation is desired.

4. *Sustaining Members:* Individuals or corporations interested in public health may be elected to Sustaining Membership. Dues $50.00 or more per year.

5. *Affiliated Societies:* A state or provincial public health association or similar regional society including more or less than a state, primarily composed of professional public health workers and organized for the same general objects as the American Public Health Association, may be elected as an Affiliated Society, provided that not less than twenty of its active members and at least one-half of its active members are Members or Fellows of the American Public Health Association. Not more than one such society shall be admitted from the same area.

A society applying for affiliation shall submit a copy of its constitution and by-laws, its last annual budget, a roster of its members and such other evidences of its qualifications as may be required. It shall submit annually and at other times such reports on its financial standing, membership and other matters as may be required by the Executive Board of the American Public Health Association.

The Committee on Fellowship and Membership shall consider all applications for affiliation and report its recommendations to the Governing Council.

The annual dues of Affiliated Societies shall be one per cent of their gross annual income, the minimum dues per society being $10.00 per year.

Each Fellow, Member, and Affiliated Member of the American Public Health Association shall be a member of the Affiliated Society to which he is eligible and no person eligible for election to an Affiliated Society shall be admitted to Membership, or Affiliated Membership in the American Public Health Association unless such person has qualified or at the same time qualifies as a member of an Affiliated Society.

For every Fellow, Member, or Affiliated Member paying annual dues to the American Public Health Association, the American Public Health Association shall remit to the Affiliated Society of which such person is a member the sum of $1.00 per annum.

6. *Affiliated Members:* This class of membership shall include all active (namely professional) members of Affiliated Societies, and in areas where no Affiliated Societies exist such other professional sanitarians as may be elected to this grade. Dues $1.00 per year.

7. *Life Members:* Upon the recommenda-

tion of the Committee on Fellowship and Membership any individual member of the Association may be elected a member for life. Election to this grade shall not affect the privileges held by such individual in his previous grade of membership. The dues for Life Members shall be $100.00, payable within one year after election, and this payment by such member shall exempt him from any further dues.

8. *Regional Branches:* The Governing Council may at its discretion establish regional branches of the Association.

B. *Election:* The election of Fellows (see A1 above), Honorary Fellows Life Members, and Affiliated Societies shall be by the Governing Council.

The election of Members and Sustaining Members shall be by the Executive Board.

Three-fourths of the votes cast shall be requisite for election.

Upon the recommendation of the Committee on Fellowship and Membership the Governing Council may discontinue the Membership, Fellowship or affiliation of any constituent. Three-fourths of the votes cast shall be necessary for such action.

C. *Dues:* Dues are payable annually in advance. All constituents paying dues shall be entitled to receive the AMERICAN JOURNAL OF PUBLIC HEALTH and, or, such other publications as may be designated by the Executive Board, which shall determine the proportion of dues to be devoted to this purpose.

Constituents of any class whose dues are unpaid for six months or more shall be considered not in good standing. Constituents not in good standing shall not be entitled to vote, hold office or enjoy other privileges or powers of membership. Good standing may be resumed upon the payment of all arrears and dues in advance for one year, provided, however, the lapsed period is not greater than one year. The Administrative Office shall notify by registered mail all constituents who have been in arrears for a period of eleven months. The names of constituents in any class whose dues remain unpaid for one year or more shall be presented to the Executive Board which shall order the names of such constituents stricken from the Membership roll, provided, however, such constituents have been duly notified as hereinbefore provided in this paragraph. Constituents whose names have been stricken from the rolls in this manner may be again admitted in the manner provided for the election of new constituents in the class for which they make application, provided such person or organization complies with the eligibility requirements at the time the new application is made.

If, in the opinion of the Executive Board, any member or Fellow of the Association be found (hereafter) to have permitted the use of his name, or otherwise to have allowed himself to be quoted or used for illustration in the advertising of a commercial product, in such a manner as to reflect discredit upon the Association, his Fellowship or Membership in the Association shall thereupon be terminated. The application of this article shall not be retroactive.

ARTICLE II GOVERNING COUNCIL

The thirty members of the Governing Council designated in Article III, Section A, Paragraph 2 of the Constitution, shall be nominated and elected as follows: There shall be a Nominating Committee composed of one Fellow elected by each Section at the preceding annual meeting, and an additional Fellow designated by the Executive Board, the latter serving as Chairman. This committee shall present to the Administrative Office at least two months before the next annual meeting the names of at least twenty and not more than thirty Fellows of the Association selected with due regard to geographical and Membership considerations as nominees for the Governing Council. The Administrative Office shall publish this list to the Membership. Upon the petition of twenty-five Fellows the Nominating Committee shall add the name of any Fellow to this list provided such petition is received fifteen days before the annual meeting. The time for closing the polls shall be determined each year by the Executive Board. The Fellows receiving the highest number of votes on a written ballot cast by the Fellows present and voting at the annual meeting shall be declared elected to fill existing vacancies. Should two or more candidates receive the same number of votes, the Executive Board shall, when necessary, determine by written ballot the order of precedence.

ARTICLE III EXECUTIVE BOARD

A. *Composition:* There shall be an Executive Board of nine members elected by the Governing Council, six of whom shall be past or present members of the Governing Council, and three of whom shall be the President, the President-elect, and the Treasurer.

1. When a Fellow accepts membership on the Executive Board, any position he may hold on any of the standing committees of the Association will thereupon automatically become vacant. This provision will become effective with the close of the annual meeting at which this amendment is adopted, at which time Fellows holding memberships on the Executive Board and any standing com-

mittees will indicate their preference for one or the other.

2. If a vacancy on the Executive Board shall occur after the annual meeting because of the preference of an elected member to retain his standing committee membership, the President shall designate a nominating committee for this purpose from the membership of the Governing Council and this committee shall nominate not less than three candidates for the vacancy, from whom the candidate receiving the highest number of votes in a mail ballot by the Governing Council shall be declared elected to the Executive Board to fill the vacancy.

B. *Terms:* The terms of the President, the President-elect, and the Treasurer as Executive Board members shall be one year each. The terms of the other members shall be three years each, expiring in rotation, two each year. The members of the Executive Board whose terms have not expired shall at the time of the adoption of this amendment continue in office until the expiration of the term for which they have been elected. At the annual meeting at which this amendment is adopted one member shall be elected for a term of two years and two members for a term of three years. Should a vacancy occur the Governing Council shall elect a member to fill such vacancy for the unexpired term. The terms of the members of the Executive Board shall begin at the end of the annual meeting at which they have been elected, and shall continue until the end of the annual meeting at expiration of term, provided that newly elected members of the Board shall have the right to attend meetings as soon as elected, and shall have no vote until installed.

C. *Reëlection:* After two consecutive terms of three years a member of the Executive Board shall be ineligible for reëlection during one Association year. This provision shall not apply to an officer of the Association.

D. *Officers:* The Executive Board shall elect from its own membership a Chairman who shall serve in that capacity for such term as the Executive Board shall determine. It shall also designate such other officers of the Board as it may require for the conduct of its business.

E. *Duties:*

1. To direct the administrative work of the Association.

2. To act as Trustee of the Association's properties.

3. To plan methods for the procurement of funds.

4. To recommend budgets for the Association's work

5. To conform to the policies of the Governing Council in the conduct of its work.

6. To appoint the members of the five Standing Committees and to authorize and confirm the appointment of all other Association committees.

7. To transmit a report of its proceedings and transactions to the Governing Council at least thirty days before each annual meeting.

8. In general to carry out the policies of the Governing Council between meetings of the latter.

F. *A Quorum* of the Executive Board shall consist of four members.

ARTICLE IV OFFICERS

The officers elected by the Governing Council shall be nominated from the floor by that body.

ARTICLE V COMMITTEES

There shall be five standing committees of the Association as follows:

1. Committee on Fellowship and Membership.
2. Committee on Meetings and Publications.
3. Committee on Administrative Practice.
4. Committee on Research and Standards.
5. Committee on Professional Education.

A. *Organization:* All of the Standing and Special Committees of the Association shall be authorized and appointed by the Executive Board unless otherwise provided for in these By-laws. Unless otherwise provided for all Section Committees shall be named by the respective Sections, and the personnel of such committees shall be confirmed by the Executive Board. The appointments of all Association and Section Committees unless otherwise provided for in these By-laws shall expire at the next annual meeting. The Chairmen of the Standing Committees shall be designated by the Executive Board. The Standing Committees shall designate from among their membership such other officers as they may require for the conduct of their business. Each committee shall control its policies within limitations prescribed by the Governing Council and the Executive Board.

The Executive Secretary shall be a member, ex-officio, of all standing Committees, and shall serve as Secretary of each such committee.

B. *Composition and Functions:*

1. The Committee on Fellowship and Membership shall consist of one Fellow to be elected by each Section and an additional Fellow elected by the Executive Board. At the time of the adoption of this amendment the Executive Board shall designate which

five of the members of this committee shall serve for one year, the balance to serve for two years. Thereafter, those designated to fill vacancies shall serve for a term of two years.

This committee shall pass upon the eligibility of Fellows, Members and other constituents, in accordance with the provisions of the By-laws. It shall carry on membership promotion and shall discharge such other duties as are assigned to it by the Governing Council or by the Executive Board.

2. The Committee on Meetings and Publications shall consist of five Fellows. At the time of adoption of this amendment the Executive Board shall elect five Fellows to serve for one, two, three, four and five years respectively. Thereafter, members shall serve for a term of five years.

This committee shall prepare plans and a program for the conduct of annual and other meetings of the Association. All publications of the Association, its Sections and committees shall be issued under the direction of this committee, which shall also be responsible for the editorial and financial policies and management of all such publications.

3. The Committee on Administrative Practice shall consist of fifteen Fellows as follows: Twelve shall be designated by the Executive Board to serve for a term of four years, the terms of three members of this committee expiring each year. The remaining three members of this Committee shall be elected annually by and from the Fellows of the Association, affiliated with the Health Officers Section.

This committee shall engage in the collection of information regarding current public health practices and analyze the material obtained to derive standards of organization and achievement. The findings and standards may be made available to public health workers through publications, information and field service under such conditions as the committee may establish. No standards shall be promulgated as the official and authorized judgment of the Association except with the approval of the Governing Council.

4. The Committee on Research and Standards shall consist of fifteen Fellows representative of the various Sections of the Association appointed by the Executive Board. In the beginning the Executive Board shall designate five who shall serve for a term of one year, five for two years, and five for three years. Thereafter members shall serve for a term of three years.

This committee shall be responsible for carrying out research and the development of standards in the technical branches of public health service, and coördinate such research and standardization. This committee shall also be charged with the duty of reviewing from time to time standards already established. No standards shall be promulgated as the official and authorized judgment of the Association except with the approval of the Governing Council.

5. The Committee on Professional Education shall consist of ten Fellows appointed by the Executive Board. At the time of the adoption of this amendment the Executive Board shall appoint ten Fellows, two to serve for one, two for two, two for three, two for four and two for five years respectively. Thereafter, members shall serve for a term of five years.

This committee shall be responsible for carrying out research and the development of standards for professional education and training in public health work and shall perform such other functions as may be delegated to the committee by the Governing Council with the view of maintaining professional qualifications of high standard. No standards shall be promulgated as the official and authorized judgment of the Association except with the approval of the Governing Council.

ARTICLE VI MEETINGS OF THE ASSOCIATION

There shall be at least one annual meeting of the Association, held at a place to be selected by the Governing Council. All papers and reports presented at the annual meeting and other meetings, including regional meetings, shall be the property of the Association for publication, unless this right is waived by the Committee on Meetings and Publications. Special meetings of the Association may be called by a majority vote of the Governing Council, the Executive Board, or the Association.

ARTICLE VII SECTIONS

The Executive Board shall approve rules and regulations relating to the government of the Sections, and to the appointment of administrative committees. Sections shall elect their own officers.

Nominating Committee: The Section Chairman with the advice of the Section Council shall appoint a Committee on Nominations at least fifteen days before each annual meeting. The Section Secretary shall be a member of such Committee.

The names of the members of the Committee on Nominations shall be announced at the first meeting of the Section, at each annual meeting of the Association. The Committee on Nominations shall present at the second meeting of the Section a list of nominees for

the Section offices, and for membership in the Section Council; provided that if the name of any Fellow be transmitted to the Nominating Committee over the signature of ten Fellows of the Section prior to the second meeting of the Section, the Nominating Committee shall add the name of such Fellow to its own list of nominees.

A. *Officers* of each Section shall be a Chairman, a Vice-Chairman, and a Secretary. The Chairman, Vice-Chairman, and Secretary shall be the representatives of the Section to the Governing Council of the Association.

B. *Terms:* New terms begin and old terms expire at the end of annual meetings. After five consecutive years in any elective Section office, except that of Secretary, a member shall be ineligible to reëlection to that office during one Association year.

C. *The Chairman* shall preside at meetings of the Section.

D. *The Vice-Chairman* shall preside at meetings of the Section in the absence of the Chairman.

E. *The Secretary* of the Section shall prepare the scientific program of the Section for the annual meeting, subject to the recommendations of the Section Council, and he shall submit same to the Committee on Meetings and Publications and shall keep the minutes, and other records of the Section, and shall transmit to the Secretary of the Association a copy of the minutes of both business and scientific sessions as soon as practicable thereafter. When unable to be present at meetings, he shall thoroughly instruct a substitute as far in advance of the meeting as possible.

F. *Section Council:* There shall be a Section Council composed of the three officers of the Section and five members, who shall be Fellows of the Section.

Terms of members of the Section Council shall be five years each. In the beginning one shall serve for one year, one for two, one for three, one for four, and one for five years.

Duties of the Section Council shall be:

1. To recommend papers, and to make general recommendations in relation to the annual meeting program.
2. To advise on Section membership.
3. To advise on Section policies.
4. To submit annually to the Governing Council through the Executive Board a report of the transactions of the Section.
5. To report annually to the Governing Council through the Executive Board on the plans, scope and policy of the Section during the succeeding year.
6. To formulate rules of procedure for the Section.
7. To approve and transmit to the Governing Council resolutions originating in the Section.
8. To advise on the publication of papers and reports presented at the Section meetings.
9. To advise with respect to the appointment of technical committees, sub-committees or Section representatives on committees of the Association.

ARTICLE VIII FINANCES

All remittances to the Association shall be deposited to the account of the Treasurer. The Treasurer shall be custodian of investments of the Association and shall disburse funds in accordance with duly authorized vouchers. With the approval of the Executive Board he may establish a drawing account for the Executive Secretary, who shall send to members of the Executive Board a financial summary of receipts and disbursements each month. Once each month, or oftener if called for, he shall also forward to the Treasurer and President an itemized statement of all expenditures. The Executive Secretary and the Treasurer shall be bonded at the expense of the Association in an amount to be determined by the Executive Board. The books of the Association shall be audited annually by certified public accountants, to be appointed by the Executive Board.

ARTICLE IX AMENDMENTS

These By-laws may be amended by a two-thirds vote of those voting on the Governing Council during the annual meeting, provided that twenty-four hours prior written notice thereof has been given. The By-laws may further be amended by a two-thirds vote of those voting at any meeting of the Governing Council called for the purpose, provided that notice thereof shall have been given at least fifteen days prior to such meeting.

THE AMERICAN PUBLIC HEALTH ASSOCIATION

50 West 50th Street, New York, N. Y.

GOVERNING COUNCIL

OFFICERS 1935–1936

President, WALTER H. BROWN, M.D., Palo Alto, Calif.
President-elect, THOMAS PARRAN, JR., M.D., Albany, N. Y.
First Vice-President, ROBERT E. WODEHOUSE, M.D., Ottawa, Ont., Can.
Second Vice-President, PROFESSOR SAMUEL C. PRESCOTT, Cambridge, Mass.
Third Vice-President, ANGEL DE LA GARZA BRITO, M.D., Mexico City, Mex.
Treasurer, LOUIS I. DUBLIN, PH.D., New York, N. Y.
Executive Secretary, REGINALD M. ATWATER, M.D., New York, N. Y.
Chairman of Executive Board, JOHN A. FERRELL, M.D., New York, N. Y.

ELECTIVE COUNCILORS

Terms Expiring 1936

WALTER H. BROWN, M.D., Palo Alto, Calif.
SAMUEL J. CRUMBINE, M.D., New York, N. Y.
A. J. DOUGLAS, M.D., Winnipeg, Man., Can.
ALLEN W. FREEMAN, M.D., Baltimore, Md.
WADE H. FROST, M.D., Baltimore, Md.
THOMAS PARRAN, JR., M.D., Albany, N. Y.
PHILIP S. PLATT, PH.D., Honolulu, T. H.
MILTON J. ROSENAU, M.D., Chapel Hill, N. C.
HENRY F. VAUGHAN, DR.P.H., Detroit, Mich.
C.-E. A. WINSLOW, DR.P.H., New Haven, Conn.

Terms Expiring 1937

DONALD B. ARMSTRONG, M.D., New York, N. Y.
J. ROSSLYN EARP, DR.P.H., Santa Fe, N. M.
J. G. FITZGERALD, Toronto, Ont., Can.
EDWARD S. GODFREY, JR., M.D., Albany, N. Y.
GUY S. MILLBERRY, D.D.S., San Francisco, Calif.
JOSEPH W. MOUNTIN, M.D., Washington, D. C.
WILLIAM H. PARK, M.D., New York, N. Y.
WILLIAM P. SHEPARD, M.D., San Francisco, Calif.
WILSON G. SMILLIE, M.D., Boston, Mass.
JOHN SUNDWALL, M.D., Ann Arbor, Mich.

Terms Expiring 1938

J. N. BAKER, M.D., Montgomery, Ala.
E. L. BISHOP, M.D., Knoxville, Tenn.
ROBERT D. DEFRIES, M.D., Toronto, Ont., Can.
JOHN A. FERRELL, M.D., New York, N. Y.
C. A. HOLMQUIST, Albany, N. Y.
JOHN F. NORTON, PH.D., Kalamazoo, Mich.
MAZYCK P. RAVENEL, M.D., Columbia, Mo.
W. FRANK WALKER, DR.P.H., New York, N. Y.
CHARLES F. WILINSKY, M.D., Boston, Mass.
ABEL WOLMAN, Baltimore, Md.

REPRESENTATIVES OF AFFILIATED SOCIETIES AND BRANCHES

L. J. DUMONT, M.D., Connecticut Public Health Association
HENRY HANSON, M.D., Florida Public Health Association
M. E. WINCHESTER, M.D., Georgia Public Health Association
CHARLES F. WILINSKY, M.D., Massachusetts Public Health Association
C. C. SLEMONS, M.D., Michigan Public Health Association
ELSBETH VAUGHAN, Missouri Public Health Association
PAUL S. FOX, C.E., New Mexico Public Health Association
_____, Northern California Public Health Association
G. D. LUMMIS, M.D., Ohio Federation of Public Health Officials
CHARLES B. CRITTENDEN, M.D., Pennsylvania Public Health Association
CHARLES W. DECKER, M.D., Southern California Public Health Association
JAMES A. HAYNE, M.D., South Carolina Public Health Association
V. M. EHLERS, Texas Public Health Association
W. BROWNLEY FOSTER, M.D., Virginia Public Health Association
JOHN THAMES, M.D., West Virginia Public Health Association
J. J. SIPPY, M.D., Western Branch
F. J. UNDERWOOD, M.D., Southern Branch

SECTION OFFICERS

Health Officers

Chm., JOHN P. KOEHLER, M.D., Milwaukee, Wis.
Vice-Chm., WILLIAM F. COGSWELL, M.D., Helena, Mont.
Secy., HUNTINGTON WILLIAMS, M.D., Baltimore, Md.
Section Council, A. H. FLICKWIR, M.D., Fort Worth, Tex., FREDERICK D. STRICKER, Portland, Ore., S. BOUCHER, M.D., Montreal, Can., LEON BANOV, M.D., Charleston, S. C., JOHN J. SIPPY, M.D., Stockton, Calif.

Laboratory

Chm., RUTH GILBERT, M.D., Albany, N. Y.
Vice-Chm., W. D. STOVALL, M.D., Madison, Wis.
Secy., FRIEND LEE MICKLE, Hartford, Conn.

Vital Statistics

Chm., GAIUS E. HARMON, M.D., Cleveland, O.
Vice-Chm., JESSAMINE S. WHITNEY, New York, N.Y.
Secy., JOHN COLLINSON, M.D., Washington, D. C.

Public Health Engineering

Chm., ARTHUR P. MILLER, C.E., New York, N. Y.
Vice-Chm., GORDON M. FAIR, Cambridge, Mass.
Secy., ROY J. MORTON, Nashville, Tenn.

Industrial Hygiene

Chm., ALBERT S. GRAY, M.D., Hartford, Conn.
Vice-Chm., LEVERETT D. BRISTOL, M.D., New York, N. Y.
Secy., BERNARD S. COLEMAN, New York, N. Y.

Food and Nutrition

Chm., FRED W. TANNER, PH.D., Urbana, Ill.
Vice-Chm., WALTER S. FRISBIE, Washington, D. C.
Secy., CARL R. FELLERS, PH.D., Amherst, Mass.

Child Hygiene

Chm., GEO. T. PALMER, DR.P.H., New York, N. Y.
Vice-Chm., A. L. BEAGHLER, M.D., Denver, Colo.
Secy., DON W. GUDAKUNST, M.D., Detroit, Mich.

Public Health Education

Chm., W. W. BAUER, M.D., Chicago, Ill.
Vice-Chm., HOMER N. CALVER, New York, N. Y.
Secy., MARY P. CONNOLLY, Detroit, Mich.

Public Health Nursing

Chm., NAOMI DEUTSCH, R.N., Berkeley, Calif.
Vice-Chm., MARY J. DUNN, R.N., Nashville, Tenn.
Secy., MARGUERITE A. WALES, R.N., New York, N.Y.

Epidemiology

Chm., KENNETH F. MAXCY, M.D., University, Va.
Vice-Chm., M. E. BARNES, M.D., Iowa City, Iowa
Secy., CLARENCE L. SCAMMAN, M.D., New York, N. Y.

Executive Board

Chairman, JOHN A. FERRELL, M.D., New York, N. Y. (1936)
J. N. BAKER, M.D., Montgomery, Ala. (1936)
JOHN P. KOEHLER, M.D., Milwaukee, Wis. (1937)
FRIEND LEE MICKLE, Hartford, Conn. (1937)
JOHN L. RICE, M.D., New York, N. Y. (1938)
HUNTINGTON WILLIAMS, M.D., Baltimore, Md. (1938)
WALTER H. BROWN, M.D., Palo Alto, Calif. (*President*)
THOMAS PARRAN, JR., M.D., Albany, N. Y. (*President-elect*)
LOUIS I. DUBLIN, PH.D., New York, N. Y. (*Treasurer*)

PAST PRESIDENTS

*Stephen Smith, M.D. 1872, 1873, 1874
*Joseph M. Toner, M.D. 1875
*Edwin M. Snow, M.D. 1876
*John H. Rauch, M.D. 1877
*Elisha Harris, M.D. 1878
*James L. Cabell, M.D. 1879
*John S. Billings, M.D. 1880
*Charles B. White, M.D. 1881
*Robert C. Kedzie, M.D. 1882
*Ezra M. Hunt, M.D. 1883
*Albert L. Gibon, M.D. 1884
*James E. Reeves, M.D. 1885
*Henry P. Walcott, M.D. 1886
*George M. Sternberg, M.D. 1887
*Charles N. Hewitt, M.D. 1888
*Hosmer A. Johnson, M.D. 1889
*Henry B. Baker, M.D. 1890
*Frederick Montizambert, M.D. 1891
*Felix Formento, M.D. 1892
*Samuel H. Durgin, M.D. 1893
*Emmanuel P. Lachapelle, M.D. 1894
*William Bailey, M.D. 1895
*Eduardo Liceaga, M.D. 1896
*Henry B. Horlbeck, M.D. 1897
*Charles A. Lindsey, M.D. 1898
*George H. Rohe, M.D. 1899
*Henry Mitchell, M.D. 1899
*Peter H. Bryce, M.D. 1900
*Benjamin Lee, M.D. 1901
*Henry D. Holton, M.D. 1902
*Walter Wyman, M.D. 1903
*Carlos J. Finlay, M.D. 1904
*Frank F. Wesbrook, M.D. 1905
*Franklin C. Robinson, LL.D. 1906
*Domingo Orvananos, M.D. 1907
*Richard H. Lewis, M.D. 1908
*Gardner T. Swarts, M.D. 1909
Charles O. Probst, M.D. 1910
R. M. Simpson, M.D. 1911
*J. N. Hurty, M.D. 1912
*Rudolph Hering, Sc.D. 1913
W. C. Woodward, M.D. 1914
*W. T. Sedgwick, Sc.D. 1915
John F. Anderson, M.D. 1916
W. A. Evans, M.D. 1917
*C. J. Hastings, M.D. 1918
*Lee K. Frankel, Ph.D. 1919
W. S. Rankin, M.D. 1920
Mazÿck P. Ravenel, M.D. 1921
A. J. McLaughlin, M.D. 1922
E. C. Levy, M.D. 1923
W. H. Park, M.D. 1924
Henry F. Vaughan, Dr.P.H. 1925
C.-E. A. Winslow, Dr.P.H. 1926
Charles V. Chapin, M.D. 1927
Herman N. Bundesen, M.D. 1928
*George W. Fuller 1929
A. J. Chesley, M.D. 1930
Hugh S. Cumming, M.D. 1931
Louis I. Dublin, Ph.D. 1932
John A. Ferrell, M.D. 1933
Haven Emerson, M.D. 1934
Eugene L. Bishop, M.D. 1935
Walter H. Brown, M.D. 1936

* Deceased.

ANNUAL MEETINGS

Preliminary	Meeting	.....	New York, N. Y., April 18	1872
"	"	.....	Long Branch, N. J., September 12	1872
1st Annual	Meeting	.....	Cincinnati, O., May 1–3	1873
2d "	"	.....	New York, N. Y., November 11–14	1873
3d "	"	.....	Philadelphia, Pa.	1874
4th "	"	.....	Baltimore, Md.	1875
5th "	"	.....	Boston, Mass.	1876
6th "	"	.....	Chicago, Ill.	1877
7th "	"	.....	Richmond, Va.	1878
8th "	"	.....	Nashville, Tenn.	1879
9th "	"	.....	New Orleans, La.	1880
10th "	"	.....	Savannah, Ga.	1881
11th "	"	.....	Indianapolis, Ind.	1882
12th "	"	.....	Detroit, Mich.	1883
13th "	"	.....	St. Louis, Mo.	1884
14th "	"	.....	Washington, D. C.	1885
15th "	"	.....	Toronto, Ont.	1886
16th "	"	.....	Memphis, Tenn.	1887
17th "	"	.....	Milwaukee, Wis.	1888
18th "	"	.....	Brooklyn, N. Y.	1889

19th	Annual Meeting	Charleston, S. C.	1890
20th	" "	Kansas City, Mo.	1891
21st	" "	Mexico City, Mex.	1892
22d	" "	Chicago, Ill.	1893
23d	" "	Montreal, Que.	1894
24th	" "	Denver, Colo.	1895
25th	" "	Buffalo, N. Y.	1896
26th	" "	Philadelphia, Pa.	1897
27th	" "	Ottawa, Ont.	1898
28th	" "	Minneapolis, Minn.	1899
29th	" "	Indianapolis, Ind.	1900
30th	" "	Buffalo, N. Y.	1901
31st	" "	New Orleans, La.	1902
32d	" "	Washington, D. C.	1903
33d	" "	Havana, Cuba	1904
34th	" "	Boston, Mass.	1905
35th	" "	Mexico City, Mex.	1906
36th	" "	Atlantic City, N. J.	1907
37th	" "	Winnipeg, Man.	1908
38th	" "	Richmond, Va.	1909
39th	" "	Milwaukee, Wis.	1910
40th	" "	Havana, Cuba	1911
41st	" "	Washington, D. C.	1912
42d	" "	Colorado Springs, Colo.	1913
43d	" "	Jacksonville, Fla.	1914
44th	" "	Rochester, N. Y.	1915
45th	" "	Cincinnati, O.	1916
46th	" "	Washington, D. C.	1917
47th	" "	Chicago, Ill.	1918
48th	" "	New Orleans, La.	1919
49th	" "	San Francisco, Calif.	1920
50th	" "	New York, N. Y.	1921
51st	" "	Cleveland, O.	1922
52d	" "	Boston, Mass.	1923
53d	" "	Detroit, Mich.	1924
54th	" "	St. Louis, Mo.	1925
55th	" "	Buffalo, N. Y.	1926
56th	" "	Cincinnati, O.	1927
57th	" "	Chicago, Ill.	1928
58th	" "	Minneapolis, Minn.	1929
59th	" "	Fort Worth, Tex.*	1930
60th	" "	Montreal, Que.	1931
61st	" "	Washington, D. C.	1932
62d	" "	Indianapolis, Ind.	1933
63d	" "	Pasadena, Calif.	1934
64th	" "	Milwaukee, Wis.	1935

* Post-Convention Meeting, Mexico City, Mex., 1930.

RECIPIENTS OF THE SEDGWICK MEMORIAL MEDAL

1929 Charles V. Chapin, M.D.
1930 Theobald Smith, M.D.*
1931 George W. McCoy, M.D.
1932 William H. Park, M.D.
1933 Milton J. Rosenau, M.D.
1934 Professor Edwin O. Jordan
1935 Haven Emerson, M.D.

* Deceased.

SECTION COUNCILS

Health Officers Section
(Organized 1908)

John P. Koehler, M.D., *Chairman,* Commissmissioner of Health, Milwaukee, Wis.
William F. Cogswell, M.D., *Vice-Chairman,* Department of Public Health, Helena, Mont.
Huntington Williams, M.D., *Secretary,* Commissioner of Health, Baltimore, Md.
A. H. Flickwir, M.D. (1940)
Frederick D. Stricker, M.D. (1939)
S. Boucher, M.D. (1938)
Leon Banov, M.D. (1937)
John J. Sippy, M.D. (1936)

Laboratory Section
(Organized 1899)

Ruth Gilbert, M.D., *Chairman,* State Department of Health, Albany, N. Y.
W. D. Stovall, M.D., *Vice-Chairman,* State Hygienic Laboratory, Madison, Wis.
Friend Lee Mickle, *Secretary,* State Department of Health Laboratories, Hartford, Conn.
W. H. Kellogg, M.D. (1940)
Robert S. Breed, Ph.D. (1939)
Anna W. Williams, M.D. (1938)
M. H. McCrady (1937)
J. V. Mulcahy (1936)

Vital Statistics Section
(Organized 1908)

Gauis E. Harmon, M.D., *Chairman,* Western Reserve University, Cleveland, Ohio
Jessamine S. Whitney, *Vice-Chairman,* 50 West 50th Street, New York, N. Y.
John Collinson, M.D., *Secretary,* Bureau of the Census, Washington, D. C.
A. W. Hedrich, Sc.D. (1940)
W. Thurber Fales, Sc.D. (1939)
Butler Toombs (1938)
John O. Spain (1937)
George H. Van Buren (1936)

Public Health Engineering Section
(Organized 1911)

Arthur P. Miller, C.E., *Chairman,* U. S. Public Health Service, Sub-Treasury Bldg., New York, N. Y.
Gordon M. Fair, *Vice-Chairman,* Harvard University, Cambridge, Mass.
Roy J. Morton, *Secretary,* State Department of Health, Nashville, Tenn.
Warren J. Scott (1940)
C. A. Holmquist (1939)
Richard Messer (1938)
George W. Putnam (1937)
V. M. Ehlers (1936)

Industrial Hygiene Section
(Organized 1914)

Albert S. Gray, M.D., *Chairman,* State Department of Health, Hartford, Conn.
Leverett D. Bristol, M.D., *Vice-Chairman,* 195 Broadway, New York, N. Y.
Bernard S. Coleman, *Secretary,* 386 Fourth Avenue, New York, N. Y.
Henry H. Kessler, M.D. (1940)
R. R. Sayers, M.D. (1939)
Louis Schwartz, M.D. (1938)
Frederick B. Flinn, Ph.D. (1937)
Alice Hamilton, M.D. (1936)

Food and Nutrition Section
(Organized 1917)

Fred W. Tanner, Ph.D., *Chairman,* University of Illinois, Urbana, Ill.
Walter S. Frisbie, *Vice-Chairman,* U. S. Food and Drug Administration, Washington, D. C.
Carl R. Fellers, Ph.D., *Secretary,* Massachusetts State College, Amherst, Mass.
D. Breese Jones, Ph.D. (1940)
Walter H. Eddy, Ph.D. (1939)
J. P. Bushong, D.V.M. (1938)
James A. Tobey, Dr.P.H. (1937)
F. C. Blanck, Ph.D. (1936)

Child Hygiene Section
(Organized 1921)

George T. Palmer, M.D., *Chairman,* Department of Health, New York, N. Y.
A. L. Beaghler, M.D., *Vice-Chairman,* 414 Fourteenth St., Denver, Colo.
Don W. Gudakunst, M.D., *Secretary,* Department of Health, Detroit, Mich.
George P. Barth, M.D. (1940)
Estella F. Warner, M.D. (1939)
Guy S. Millberry, M.D. (1938)
Elizabeth Gardiner, M.D. (1937)
Murray P. Horwood (1936)

Public Health Education Section
(Organized 1922)

W. W. Bauer, M.D., *Chairman,* 535 N. Dearborn Street, Chicago, Ill.
Homer N. Calver, *Vice-Chairman,* 50 West 50th Street, New York, N. Y.
Mary P. Connolly, *Secretary,* 3919 John R Street, Detroit, Mich.
Marjorie Delavan (1940)

William P. Shepard, M.D. (1939)
Evart G. Routzahn (1938)
Ira V. Hiscock (1937)
C. E. Turner (1936)

PUBLIC HEALTH NURSING SECTION
(Organized 1923)

Naomi Deutsch, R.N., *Chairman,* U. S. Public Health Service, Washington, D. C.
Mary J. Dunn, *Vice-Chairman,* Vanderbilt University, Nashville, Tenn.
Marguerite A. Wales, R.N., *Secretary,* 99 Park Avenue, New York, N. Y.
Pearl McIver, R.N. (1940)
Marion W. Sheahan (1939)
Anna Heisler, R.N. (1938)
Ruth Houlton, R.N. (1937)
Amelia Grant, R.N. (1936)

EPIDEMIOLOGY SECTION
(Organized 1929)

Kenneth F. Maxcy, M.D., *Chairman,* University of Virginia, University, Va.
M. E. Barnes, M.D., *Vice-Chairman,* University of Iowa, Iowa City, Ia.
Clarence L. Scamman, M.D., *Secretary,* Commonwealth Fund, New York, N. Y.
J. A. Doull, M.D., (1940)
James P. Leake, M.D. (1939)
C. D. Barrett, M.D. (1938)
Haven Emerson, M.D. (1937)
Alton S. Pope, M.D. (1936)

ASSOCIATE EDITORS OF THE JOURNAL

HENRY F. VAUGHAN, Dr.P.H., Health Officers
JOHN F. NORTON, Ph.D., Laboratory
ARTHUR W. HEDRICH, Sc.D., Vital Statistics
ARTHUR P. MILLER, C.E., Public Health Engineering
HENRY H. KESSLER, M.D., Industrial Hygiene
WALTER S. FRISBIE, Food and Nutrition
RICHARD A. BOLT, M.D., Dr.P.H., Child Hygiene
EVART G. ROUTZAHN, Public Health Education
KATHERINE E. FAVILLE, R.N., Public Health Nursing
KENNETH F. MAXCY, M.D., Dr.P.H. Epidemiology

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Katherine Andrus, Chief Clerk
Jeanne L. Bickel, Bookkeeper
Madeleine Fenning, Stenographer
John Fay, Clerk

Committee on Fellowship and Membership
Elsie A. Siemer, Secretary of Committee
Fannie Silver, Membership Clerk
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Chemical Methods of Sewage—F. Wellington Gilcreas, State Department of Health, Albany, N. Y.

Microscopic Methods of Water and Sewage—Theodore A. Olsen, Division of Sanitation, State Board of Health, Minneapolis, Minn.

Swimming Pools and Bathing Place Waters—W. L. Mallmann, Ph.D., Dept. of Bacteriology and Hygiene, Michigan State College, East Lansing, Mich., and M. S. Nichols, Ph.D., State Department of Health, Madison, Wis.

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Methods of Detecting Specific Types of Bacteria in Dairy Products—Mac H. McCrady, 89 Notre Dame East, Montreal, Que., Canada

Shellfish Examinations—C. A. Perry, Sc.D., Bureau of Bacteriology, State Dept. of Health, Baltimore, Md.

Laboratory Equipment—R. V. Stone, D.V.M., County Health Department, Los Angeles, Calif.

Methods for Detecting Organisms of Colon Group—A. J. Slack, M.D., Institute of Public Health, London, Ontario, Canada

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Committee on Progress in and Present Status of Biological Products (allocated to Committee on Research and Standards)

William H. Park, M.D., *Chairman*, Foot of East 16th Street, New York, N. Y.
Donald T. Fraser, M.B.
George W. McCoy, M.D.
Elliott S. A. Robinson, M.D., Ph.D.
A. B. Wadsworth, M.D.

Committee on Advisability of Laboratory Examination of Food Handlers (allocated to Committee on Research and Standards)

Minna Crooks Young, *Chairman*, State Department of Health, Lansing, Mich.
Ruth Gilbert, M.D.
S. A. Koser, Ph.D.
A. L. MacNabb, D.V.M.
Friend Lee Mickle
Mazÿck P. Ravenel, M.D.

Committee on Waterways Pollution Studies (to coöperate with Committee on Waterways Pollution of Public Health Engineering Section)

James A. Newlands, *Chairman*, 11 Laurel Street, Hartford, Conn.
E. J. Theriault

Committee on Section Representation on the Governing Council (unallocated)

Friend Lee Mickle, *Chairman*, Box 1139, Hartford, Conn.
Robert S. Breed, Ph.D.
Ruth Gilbert, M.D.
W. H. Kellogg, M.D.
Mazÿck P. Ravenel, M.D.
W. D. Stovall, M.D.

Committee on Scientific Exhibits (allocated to Committee on Meetings and Publications)

Fred O. Tonney, M.D., *Chairman*, City Board of Health, Chicago, Ill.
W. D. Dotterer
Ruth Gilbert, M.D.
A. Parker Hitchens, M.D.
A. L. MacNabb, D.V.M.

Committee on Aspects of Veterinary Public Health (allocated to Committee on Meetings and Publications)

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William A. Hagan, D.V.M.
John G. Hardenbergh, D.V.M.
A. L. MacNabb, D.V.M.
I. A. Merchant, D.V.S.

Laboratory Section Representative on the Commission for the Study of Biological Stains

W. D. Stovall, M.D., State Laboratory of Hygiene, Madison, Wis.

Vital Statistics Section

Committee on Accident Statistics (allocated to Committee on Administrative Practice)

Robert J. Vane, *Acting Chairman*, Metropolitan Life Insurance Company, 1 Madison Avenue, New York, N. Y.
Earle G. Brown, M.D.
J. V. DePorte, Ph.D.
W. Thurber Fales, Sc.D.
T. F. Murphy, M.D.
Irva C. Plummer
George H. Van Buren
*R. L. Forney (Consultant)

Committee to Revise the Rules of Statistical Practice (allocated to Committee on Administrative Practice)

Timothy F. Murphy, M.D., *Chairman*, Bureau of Census, Washington, D. C.
Halbert L. Dunn, M.D., Ph.D.
J. V. DePorte, Ph.D.

* Not a member of A.P.H.A.

Committee on Forms and Methods of Statistical Practice (allocated to Committee on Administrative Practice)

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Selwyn D. Collins, Ph.D.
John Collinson, M.D., Dr.P.H.
*Sheldon L. Howard
L. W. Hutchcroft
Timothy F. Murphy, M.D.
Elizabeth Parkhurst

Committee on Registration of Births out of Wedlock (allocated to Committee on Administrative Practice)

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W. Thurber Fales, Sc.D.
*Sybil Foster
*Agnes K. Hanna
L. W. Hutchcroft

Committee on Death Certification (allocated to Committee on Administrative Practice)

Marjorie T. Bellows, *Chairman*, Department of Health, Westchester County, White Plains, New York
Haven Emerson, M.D.
Matthias Nicoll, Jr., M.D.
The members of the Vital Statistics Section Council

Committee on Exhibits (allocated to Committee on Meetings and Publications)

John Collinson, M.D., Dr.P.H., *Chairman*, Bureau of the Census, Washington, D. C.

Public Health Engineering Section

Committee on Bathing Places (Joint Committee with Conference of State Sanitary Engineers) (allocated to Committee on Research and Standards)

W. J. Scott, *Chairman*, 947 West Boulevard, Hartford, Conn.
J. L. Barron
W. H. Cary
S. DeM. Gage

Advisers

Epidemiology Section

L. L. Arnold, M.D.
M. J. Rosenau, M.D.

Laboratory Section

W. L. Mallmann, Ph.D.
M. S. Nichols, Ph.D.

Committee on Air Hygiene (allocated to the Committee on Research and Standards)

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S. DeM. Gage
H. B. Moller
Edward Wright

Advisers

Philip Drinker
F. O. Tonney, M.D.

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E. B. Besselievre
G. M. Fair
E. L. Filby
A. H. Fletcher
R. F. Goudey
H. B. Hommon
C. G. Hyde
C. E. Keefer
T. J. Lafreniere
Richard Messer
W. J. Orchard
E. L. Waterman
L. F. Warrick
R. S. Weston

Committee on Industrial Sanitation (unallocated)

C. L. Pool, *Chairman*, Room 335, State Office Bldg., Providence, R. I.
W. Scott Johnson
W. J. Scott

Committee on Milk Supply (Joint Committee with Conference of State Sanitary Engineers) (allocated to Committee on Research and Standards)

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H. A. Whittaker

Committee on Municipal Public Health Engineering (unallocated)

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Aime Cousineau
A. H. Fletcher
A. E. Gorman
W. S. Johnson
F. G. Legg
Sol Pincus

* Not a member of A.P.H.A.

Committee on Plumbing (Joint Committee with Conference of State Sanitary Engineers) (allocated to Committee on Research and Standards)

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H. E. Babbitt
W. C. Groeniger
A. E. Hansen
H. N. Old

Committee on Promotion of Environmental Sanitation (allocated to Committee on Research and Standards)

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Earl Devendorf
C. G. Gillespie
H. A. Kroeze
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Jane H. Rider

Committee on Rural Sanitation (Joint Committee with Conference of State Sanitary Engineers and the American Society of Agricultural Engineers) (allocated to Committee on Research and Standards)

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L. H. Male
A. H. Wieters

Committee on Scope of Technical Activities (unallocated)

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G. M. Fair (1939)
P. S. Fox (1940)
A. E. Gorman (1937)
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Committee on Shellfish (allocated to Committee on Research and Standards)

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Edward Wright

Adviser

C. T. Butterfield

Committee on Summer Camps and Roadside Places Involving Health Hazards

E. D. Rich, *Chairman*, State Health Dept., Lansing, Mich.
C. E. Green
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Committee on Waterways Pollution (allocated to Committee on Research and Standards)

L. F. Warrick, *Chairman*, Capitol Building, Madison, Wis.
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R. M. Harris
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Advisers

Epidemiology Section
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M. V. Veldee, M.D.
Laboratory Section
James A. Newlands

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Industrial Hygiene Section

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Henry Field Smyth, M.D., Dr.P.H., *Chairman*, University of Pennsylvania, Philadelphia, Pa.
*Volney S. Cheney, M.D.

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* Not a member of the A.P.H.A.

Committee on Standard Practices in the Problem of Compensation of Occupational Diseases (allocated to Committee on Research and Standards)

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Emery R. Hayhurst, M.D.
May R. Mayers, M.D.
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R. R. Sayers, M.D.

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Committee on Skin Irritants (allocated to Committee on Research and Standards)

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Leonard Greenburg, M.D.
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Carey P. McCord, M.D.

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* Not a member of the A.P.H.A.

Food and Nutrition Section

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John W. M. Bunker, Ph.D.
William E. Krauss, Ph.D.
Robert W. Pilcher, Ph.D.
Henry T. Scott, Ph.D.
George C. Supplee, Ph.D.

Committee on Participation in Standard Methods (allocated to Committee on Research and Standards)

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Committee on Nutritional Problems (allocated to Committee on Research and Standards)

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Conrad A. Elvehjem, Ph.D.
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Ferdinand A. Korff
Merrill J. Mack
Margaret C. Smith, Ph.D.
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Committee on Foods (except milk) (allocated to Committee on Research and Standards)

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Margaret R. Sandels, Ph.D.
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Public Health Education Section

Committee on Methods in Health Education (unallocated)

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Homer N. Calver
Mary P. Connolly
Iago Galdston, M.D.
Elizabeth Nickerson
Evart G. Routzahn
William P. Shepard, M.D.
C. E. Turner, Dr.P.H.

Public Health Nursing Section

Editorial Committee (allocated to Committee on Meetings and Publications)

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Helen Bond, R.N.
Rachel K. Miller, R.N.
Eva F. MacDougall, R.N.

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I. Malinde Havey, R.N., *Chairman*, 3415 Morrison Street, N.W., Washington, D. C.
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Alma Haupt, R.N.
Ruth Houlton, R.N.

Committee to Study Public Health Nursing in State Health Departments in Coöperation with the National Organization for Public Health Nursing (allocated to Committee on Administrative Practice)

Marion Sheahan, R.N., *Chairman*, State Bureau of Public Health Nursing, Albany, N. Y.
Margaret East, R.N.
Alma Haupt, R.N. (representing the N.O.P.H.N.)
Eleanor Kennedy, R.N.
Olivia Peterson, R.N.

Epidemiology Section

Committee on Bathing Places (to coöperate with Committee on Bathing Places of Public Health Engineering Section)

M. J. Rosenau, M.D.
L. L. Arnold, M.D.

Committee on Waterways Pollution (to cooperate with Committee on Waterways Pollution of Public Health Engineering Section)

Milton V. Veldee, M.D.
Edwin O. Jordan, M.D.

AFFILIATED STATE PUBLIC HEALTH SOCIETIES

CONNECTICUT PUBLIC HEALTH ASSOCIATION. Affiliated with the American Public Health Association 1928. Object—To protect and promote public health and safety. Officers—President, B. B. Robbins, M.D.; Vice-President, L. J. Dumont, M.D.; Secretary-Treasurer, Professor Ira V. Hiscock.

FLORIDA PUBLIC HEALTH ASSOCIATION, INC. Affiliated with the American Public Health Association 1932. Objects—To assist in protecting and promoting public health, to provide for scientific advancement of members, and to extend and develop the public health movement. Officers—President, T. H. D. Griffitts, M.D.; First Vice-President, W. E. Van Landingham, M.D.; Second Vice-President, Russell Broughman; Secretary-Treasurer, Stewart G. Thompson, D.P.H.

GEORGIA PUBLIC HEALTH ASSOCIATION. Affiliated with the American Public Health Association 1930. Objects—To assist in protecting public health, to provide for scientific advancement of members, and to extend and develop the public health movement. Officers—President, Dr. Hugo Robinson; Secretary, Millard E. Winchester, M.D.

MASSACHUSETTS PUBLIC HEALTH ASSOCIATION. Affiliated with the American Public Health Association 1925. Objects—The advancement of sanitary science in the State of Massachusetts, the promotion of better organization and coöperation in the local Boards of Health, the uniform enforcement of sanitary laws and regulations and the establishment of pleasant social relations among members of the Association. Officers—President, Paul R. Withington, M.D.; First Vice-President, Ernest M. Morris, M.D.; Second Vice-President, Professor Curtis M. Hilliard; Secretary-Treasurer, G. Donald Buckner.

MICHIGAN PUBLIC HEALTH ASSOCIATION. Affiliated with the American Public Health Association 1922. Object—To promote the interest of public health in Michigan. Officers—President, Garner M. Byington, M.D.; Vice-President, J. D. Brook, M.D.; Secretary-Treasurer, Marjorie Delavan.

MISSOURI PUBLIC HEALTH ASSOCIATION. Affiliated with the American Public Health Association 1928. Objects—To assist in protecting and promoting public health; to provide for scientific advancement of its members, and to extend and develop the public health movement in Missouri. Officers—President, J. F. Bredeck, M.D., First Vice-President, Dr. W. A. Norris; Second Vice-President, Elnore Hackman; Secretary, C. F. Adams, M.D.; Treasurer, Joe Kruegel.

NEW MEXICO PUBLIC HEALTH ASSOCIATION. Affiliated with the American Public Health Association 1925. Object—To encourage the advancement of public health work in the state. Officers—President, Dr. Eugene P. Sims; Vice-President, Dr. Walter E. Kase; Secretary-Treasurer, Paul S. Fox, C.E.

NORTHERN CALIFORNIA PUBLIC HEALTH ASSOCIATION. Affiliated with the American Public Health Association 1927. Objects—To aid in the promotion and protection of public health; to provide for scientific advancement of members; and to extend and develop the public health movement; to aid in the promulgation and enforcement of state and local legislation in the interest of public health; to render service and volunteer trained assistance in times of stress or need to state or local health authorities. Officers—President, J. C. Geiger, M.D.; President-Elect, E. H. Coleman, M.D.; Vice-President, Samuel H. Greene; Secretary, I. O. Church, M.D.; Treasurer, Helen Hartley, R.N.

OHIO FEDERATION OF PUBLIC HEALTH OFFICIALS. Affiliated with the American Public Health Association 1927. Objects—To unite the official Public Health Employees of Ohio for educational, social and other beneficial endeavors; to encourage and promote improvement in public health service and in the status of public health employees in the State of Ohio; to encourage and promote a more active and favorable public interest in public health; to coöperate with other organizations and groups in promoting and furthering like objects; to be the official Ohio Section of the American Public Health Association. Officers—President, W. G. Rhoten, M.D.; Vice-President, A. G. Sturgiss, M.D.; Secretary-Treasurer, W. D. Bishop, M.D.

PENNSYLVANIA PUBLIC HEALTH ASSOCIATION. Affiliated with the American Public Health Association 1925. Object—To protect and promote public and personal health in Pennsylvania. Officers—President, James R Smith, M.D.; First Vice-President, Thomas Henderson, M.D.; Second Vice-President, Harry A. Fritschman; Executive Secretary, J. Clarence Funk; Treasurer, A. J. Bohl; Assistant Treasurer, Arthur M. Dewees.

SOUTH CAROLINA PUBLIC HEALTH ASSOCIATION. Affiliated with the American Public Health Association 1928. Object—To

protect and promote public health in South Carolina. Officers—President, Dr. B. M. Wyman; Vice-President, Dr. W. A. Carrigan; Secretary-Treasurer, Laura Blackburn, R.N.

SOUTHERN CALIFORNIA PUBLIC HEALTH ASSOCIATION. Affiliated with the American Public Health Association 1927. Objects—To assist in the protection and promotion of public health; to provide for the scientific advancement of its members; to extend and develop the public health movement. Officers—President, E. B. Godfrey, M.D.; President-Elect, R. V. Stone, M.D.; First Vice-President, R. C. Main, M.D.; Second Vice-President, W. F. Fox, M.D.; Secretary-Treasurer, T. P. B. Jones.

TEXAS PUBLIC HEALTH ASSOCIATION. Affiliated with the American Public Health Association 1925. Objects—To promulgate for public health workers courses of instruction and the general advancement of knowledge for sanitary improvements and for their encouragement; to provide social intercourse among its members and a friendly exchange of information and experiences. Officers—President, F. R. Tucker, M.D.; First Vice-President, Dr. Burke Brewster; Second Vice-President, Edward Robinson; Third Vice-President, Mrs. Ida Levinson; Fourth Vice-President, H. E. Hargis; Secretary-Treasurer, Lewis Bracy.

VIRGINIA PUBLIC HEALTH ASSOCIATION. Affiliated with the American Public Health Association 1925. Object—To promote the public health and all objects of the American Public Health Association. Officers—President, B. B. Bagby, M.D.

WEST VIRGINIA PUBLIC HEALTH ASSOCIATION. Affiliated with the American Public Health Association 1927. Object—To promote public and personal health, especially in West Virginia. Officers—President, A. J. Kemper, M.D.; First Vice-President, W. G. C. Hill, M.D.; Second Vice-President, R. M. Pedicord, M.D.; Secretary-Treasurer, John Thames, M.D.

WESTERN BRANCH, AMERICAN PUBLIC HEALTH ASSOCIATION

Affiliated with the American Public Health Association 1930. Object: To protect and promote public and personal health, and to promote better health service in the western portions of the United States and the Dominion of Canada, and in certain islands of the Pacific. Membership consists of the constituents of all classes of the American Public Health Association in the following territory: Arizona, California, Colorado, Idaho, Montana, Nevada, New Mexico, Oregon, Utah, Washington, Wyoming, Alaska, Hawaii, Philippine Islands, British Columbia (Canada). Officers—President, W. F. Cogswell, M.D.; President-Elect, H. E. Young, M.D.; First Vice-President, J. D. Dunshee, M.D.; Second Vice-President, J. L. Jones, M.D.; Third Vice-President, W. H. Kellogg, M.D.; Secretary, W. P. Shepard, M.D.; Treasurer, W. F. Higby.

Meetings: 1st Annual Meeting—Salt Lake City, Utah—June 12–14, 1930; 2d Annual Meeting—Seattle, Wash.—May 28–30, 1931; 3d Annual Meeting—Denver, Colo.—June 2–4, 1932; 4th Annual Meeting—Pasadena, Calif.—May 29–31, 1933; 5th Annual Meeting held in conjunction with the Sixty-Third Annual Meeting of the American Public Health Association in Pasadena, Calif., September 3–6, 1934; 6th Annual Meeting—Helena, Montana—July 1–4, 1935; 7th Annual Meeting—Vancouver, B. C.—June 24–27, 1936.

SOUTHERN BRANCH, AMERICAN PUBLIC HEALTH ASSOCIATION

Affiliated with the American Public Health Association 1932. Object—The closer bringing together of public health workers for the purpose of fostering and stimulating a greater degree of scientific effort in the discharge of their duties in the protection and promotion of public and personal health. Membership is limited to persons who are members of the American Public Health Association and may be drawn from Alabama, Arkansas, Florida, Georgia, Kentucky, Louisiana, Maryland, Mississippi, Missouri, North Carolina, Oklahoma, South Carolina, Texas, Virginia, West Virginia, and Tennessee; and from the District of Columbia, Mexico, Cuba, Puerto Rico and such other territory as includes membership in the American Public Health Association in the tropics and sub-tropics.

Officers—President, I. C. Riggin, M.D.; First Vice-President, C. J. Vaughn, M.D.; Second Vice-President, Frances Montgomery, R.N.; Third Vice-President, M. Z. Bair; Secretary-Treasurer, G. Foard McGinnes, M.D.

Meetings: 1st Annual Meeting—Birmingham, Ala.—November 14–16, 1932; 2nd Annual Meeting, Richmond, Va.—November 13, 1933; 3rd Annual Meeting, San Antonio, Tex.—November 12–14, 1934; 4th Annual Meeting—St. Louis, Mo.—November 19–20, 1935; 5th Annual Meeting—Baltimore, Md.—November, 1936.

Resolutions

THE Chairman of the Resolutions Committee, John A. Ferrell, M.D., read the following resolutions, which were unanimously adopted by the Governing Council, October 9, 1935.

I. IN MEMORIAM

RESOLVED, that it is with a sense of irreparable loss that the American Public Health Association records the deaths, since our last Annual Meeting, of the following fifty members and Fellows:

H. L. Abramson, M.D., St. John, N. B., Canada. Elected Member 1919

H. L. Akridge, M.D., Brunswick, Ga. Elected Member 1923, Fellow 1929

Lemar W. Andrews, M.D., Warsaw, N. Y. Elected Member 1919

C. A. Bevan, M.D., West Haven, Conn. Elected Member 1918, Fellow 1923

George H. Bigelow, M.D., Boston, Mass. Elected Member 1923, Fellow 1929

Dr. John H. Blanks, Zion, Ill. Elected Member 1925

J. A. Chevigny, M.D., Hammond, Ind. Elected Member 1931

Marguerite J. Clancy, R.N., Asbury Park, N. J. Elected Member 1926

E. Linwood Cornman, V.M.D., Marietta, Pa. Elected Member 1926

Henry A. Cotton, M.D., Trenton, N. J. Elected Member 1932

Nelson C. Davis, M.D., Bahia, Brazil, S. A. Elected Member 1923

F. F. DeVore, M.D., Toledo, O. Elected Member 1927

C. St. Clair Drake, M.D., Jacksonville, Ill. Elected Member 1914

Francis C. Driscoll, A.B., Quincy, Mass. Elected Member 1934

Carroll W. Eddy, D.V.M., Cleveland, O. Elected Member 1926

M. Frances Etchberger, Baltimore, Md. Elected Member 1931

Harry F. Ferguson, Springfield, Ill. Elected Member 1916

Frederick T. Fitch, M.D., East Hampton, Conn. Elected Member 1932

M. B. Harutun, M.D., Joplin, Mo. Elected Member 1920, Fellow 1923

M. O. Heckard, M.D., Chicago, Ill. Elected Member 1907, Fellow 1922

W. G. Hollingworth, D.V.S., Utica, N. Y. Elected Member 1919, Fellow 1926

John C. Humphreys, M.D., Philadelphia, Pa. Elected Member 1926

George W. Hunter, Ph.D., Claremont, Calif. Elected Member 1928

George H. Jennings, M.D., Jewett City, Conn. Elected Member 1916

J. H. Kuser, M.D., San Rafael, Calif. Elected Member 1920

John LeFeber, Milwaukee, Wis. Elected Member 1913

George T. Lennon, Haverhill, Mass. Elected Member 1918

Jorge Le-Roy, M.D., Havana, Cuba. Elected Member 1911, Fellow 1923

Charles W. Many, M.D., Doylestown, Pa. Elected Member 1917

Mae E. Mathers, R.N., Ashland, Va. Elected Member 1934

Lucy Minnigerode, R.N., Washington, D. C. Elected Member 1924

Paul F. Nichols, M.S., Berkeley, Calif. Elected Member 1934

Charles Norris, M.D., New York, N. Y. Elected Member 1920

Herbert C. Ober, M.D., D.M.D., Newton, Mass. Elected Member 1932

Dr. James M. Parrott, Raleigh, N. C. Elected Member 1932

Mrs. William L. Putnam, Boston, Mass. Elected Member 1915

Vernon Robins, M.D., Louisville, Ky. Elected Member 1916, Fellow 1922

Charles V. Roman, M.D., Nashville, Tenn. Elected Member 1926

Mrs. Joseph Sanders, Washington, D. C. Elected Member 1933

Anna M. Scholfield, Providence, R. I. Elected Member 1932

Kathryn Schulken, R.N., Denver, Colo. Elected Member 1931, Fellow 1934

G. H. Sherman, M.D., Detroit, Mich. Elected Member 1918

Sir Clifford Sifton, Toronto, Ont., Canada. Honorary Fellow

Thomas J. Strauch, Richmond, Va. Elected Member 1919

Charles Strauss, New York, N. Y. Elected Member 1914

George R. Thompson, M.D., Luzerne, N. Y. Elected Member 1931

A. Brioso Vasconcelos, M.D., Mexico City,

Mex. Elected Member 1921, Fellow 1923
William H. Wakelee, Southbury, Conn. Elected Member 1933
Henry P. Walcott, M.D., Cambridge, Mass. Honorary Fellow
Prof. John Weinzirl, Seattle, Wash. Elected Member 1906, Fellow 1922

II. ENVIRONMENTAL SANITATION

WHEREAS there exist along the boundary between Mexico and the United States various important health problems, including especially those relating to environmental sanitation, and

WHEREAS the solution of these problems involves the concerted efforts of the public health authorities of both countries and of the states concerned, and

WHEREAS plans for dealing with these problems should be coöperatively formulated and effectively carried out, therefore be it

RESOLVED, that the American Public Health Association is in sympathy with the objectives stated, and that its President is authorized in his discretion to appoint a committee to coöperate with the health authorities, federal, and state, of both countries, in formulating a suitable program and in carrying it out.

III. RESOLUTION TO LOCAL COMMITTEE AND OTHER LOCAL GROUPS

RESOLVED, that the officers and members of the American Public Health Association present at its 64th Annual Meeting in Milwaukee, Wis., express their deep appreciation to the Mayor, the Common Council, the City Departments and the citizenry of Milwaukee for their warm reception and generous hospitality; and be it further

RESOLVED, that the gratitude of the officers and members be extended to Dr. John P. Koehler, Health Commissioner of Milwaukee, and General Chairman of the Local Committee, to every member of his committee, and to all organizations represented thereon, and to the Wisconsin State Board of Health, under the direction of Dr. C. A. Harper, for their many courtesies and efficient helpfulness and generosity in their provisions for this meeting, and be it further

RESOLVED, that the sincere admiration and thanks of the assembled membership be recorded to Dr. John P. Koehler, and to every one of his assistants and helpers for their devoted attention to the planning and conduct of this meeting, which will be long remembered as one of the most successful in the Association's history.

IV. RESOLUTION TO PRESS AND RADIO

RESOLVED, that the American Public Health Association acknowledge its indebtedness to the press and the radio, national, state, and local, for their excellence of service in connection with its 64th Annual Meeting.

V. RESOLUTION TO MILWAUKEE HOTELS

RESOLVED, that the American Public Health Association record its appreciation to the Milwaukee Association of Commerce, the Milwaukee Auditorium, and the Hotel Schroeder and other Milwaukee hotels, for their valuable assistance in the conduct of its 64th Annual Meeting.

VI. RESOLUTION TO EXECUTIVE OFFICE STAFF AND COMMITTEE ON MEETINGS AND PUBLICATIONS

RESOLVED, that the commendation of the officers and the members be recorded to the Executive Office Staff of the American Public Health Association, particularly with reference to the Committee on Meeting and Publications, for efficient service in connection with the 64th Annual Meeting.

The Health Conservation Contests

SINCE 1929 the Chamber of Commerce of the United States with the coöperation of the American Public Health Association has conducted annually a Health Conservation Contest for cities. The success of these competitions was so outstanding that in 1934 a similar Contest for rural areas was inaugurated.

The names of the first award and honorable mention winners in both Contests since their inception are listed below:

1929 CITY HEALTH CONTEST AWARDS *

Group I:
Winning City: Milwaukee, Wis.
Honorable Mention Cities: Detroit, Mich., Philadelphia, Pa., San Francisco, Calif., Brooklyn, N. Y., and Buffalo, N. Y.

Group II:
Winning City: Syracuse, N. Y.
Honorable Mention Cities: New Haven, Conn., Rochester, N. Y., Cincinnati, Ohio, Yonkers, N. Y., and Albany, N. Y.

Group III:
Winning City: East Orange, N. J.
Honorable Mention Cities: Rockford, Ill., Racine, Wis., Pasadena, Calif., Harrisburg, Pa., and Greensboro, N. C.

Group IV:
Winning City: White Plains, N. Y.
Honorable Mention Cities: Cumberland, Md., Alhambra, Calif., Aurora, Ill., Durham, N. C., and Santa Ana, Calif.

Group V:
Winning City, Sidney, Ohio
Honorable Mention Cities: LaSalle, Ill., South Orange, N. J., Eureka, Kans., Palo Alto, Calif., and Natchitoches, La.

1930 CITY HEALTH CONTEST AWARDS †

Group I:
Winning City: Detroit, Mich.
Honorable Mention Cities: Milwaukee, Wis., Philadelphia, Pa., San Francisco, Calif., Baltimore, Md., and Buffalo, N. Y.

Group II:
Winning City: Newark, N. J.
Honorable Mention Cities: Cincinnati, Ohio, Rochester, N. Y., Kansas City, Mo., Denver, Colo., and Memphis, Tenn.

Group III:
Winning City: New Haven, Conn.
Honorable Mention Cities: Syracuse, N. Y., Yonkers, N. Y., Hartford, Conn., Utica, N. Y., and Reading, Pa.

Group IV:
Winning City: Racine, Wis.
Honorable Mention Cities: Harrisburg, Pa., Evanston, Ill., East Orange, N. J., Durham, N. C., and Pasadena, Calif.

Group V:
Winning City: Alhambra, Calif.
Honorable Mention Cities: Salem, Ore., White Plains, N. Y., West Orange, N. J., Watertown, N. Y., and Fargo, N. D.

Group VI:
Winning City: Chestertown, Md.
Honorable Mention Cities: Sidney, Ohio, South Orange, N. J., Albany, Ga., Palo Alto, Calif., and LaSalle, Ill.

1931 CITY HEALTH CONTEST AWARDS †

Group I:
Winning City: Milwaukee, Wis.
Honorable Mention Cities: Baltimore, Md., Detroit, Mich., Philadelphia, Pa., Pittsburgh, Pa., and St. Louis, Mo.

Group II:
Winning City: Rochester, N. Y.
Honorable Mention Cities: Cincinnati, Ohio, Kansas City, Mo., Minneapolis, Minn., Newark, N. J., and Toledo, Ohio

Group III:
Winning City: New Haven, Conn.
Honorable Mention Cities: Grand Rapids, Mich., Hartford, Conn., Syracuse, N. Y., Utica, N. Y., and Yonkers, N. Y.

Group IV:
Winning City: Evanston, Ill.
Honorable Mention Cities: East Orange, N. J., Harrisburg, Pa., Kenosha, Wis., Pasadena, Calif., and Racine, Wis.

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* Group I—Cities of over 500,000 population
" II—Cities of 100,000 to 500,000 population
" III—Cities of 50,000 to 100,000 population
" IV—Cities of 20,000 to 50,000 population
" V—Cities under 20,000 population

† Group I—Cities of over 500,000 population
" II—Cities of 250,000 to 500,000 population
" III—Cities of 100,000 to 250,000 population
" IV—Cities of 50,000 to 100,000 population
" V—Cities of 20,000 to 50,000 population
" VI—Cities under 20,000 population

Group V:
Winning City: Brookline, Mass.
Honorable Mention Cities: Alhambra, Calif., Maplewood, N. J., Newburgh, N. Y., Orange, N. J., West Orange, N. J., and Watertown, N. Y.
Group VI:
Winning City: LaSalle, Ill.
Honorable Mention Cities: Chestertown, Md., Lodi, Calif., Monrovia, Calif., Palo Alto, Calif., and Shorewood, Wis.

1932 CITY HEALTH CONTEST AWARDS †

Group I:
Winning City: Detroit, Mich.
Honorable Mention Cities: Milwaukee, Wis., Chicago, Ill., Baltimore, Md., Pittsburgh, Pa., and Buffalo, N. Y.
Group II:
Winning City: Cincinnati, Ohio
Honorable Mention Cities: Rochester, N. Y., Newark, N. J., Toledo, Ohio, Minneapolis, Minn., and Dallas, Tex.
Group III:
Winning Cities: Syracuse, N. Y., and New Haven, Conn. (Tied)
Honorable Mention Cities: Peoria, Ill., Hartford, Conn., Grand Rapids, Mich., Reading, Pa., and Yonkers, N. Y.
Group IV:
Winning City: East Orange, N. J.
Honorable Mention Cities: Evanston, Ill., Pasadena, Calif., Schenectady, N. Y., Pittsfield, Mass., Charleston, S. C., and Lincoln, Nebr.
Group V:
Winning City: Brookline, Mass.
Honorable Mention Cities: Santa Barbara, Calif., West Hartford, Conn., Newburgh, N. Y., Watertown, N. Y., and Santa Ana, Calif.
Group VI:
Winning City: Lodi, Calif.
Honorable Mention Cities: Chestertown, Md., Shorewood, Wis., Palo Alto, Calif., LaSalle, Ill., and Peru, Ill.

1933 CITY HEALTH CONTEST AWARDS †

Special Award Cities (Those cities which have twice won First Award and in 1933 have maintained their previous high standards): Detroit, Mich., Milwaukee, Wis., Syracuse, N. Y., New Haven, Conn., and Brookline, Mass.

Group I:
Winning City: Baltimore, Md.
Honorable Mention Cities: Chicago, Ill., and Pittsburgh, Pa.
Group II:
Winning City: Rochester, N. Y.
Honorable Mention Cities: Cincinnati, Ohio, Oakland, Calif., Newark, N. J., Toledo, Ohio, Kansas City, Mo., and Dallas, Tex.
Group III:
Winning City: Hartford, Conn.
Honorable Mention Cities: Grand Rapids, Mich., Duluth, Minn., Reading, Pa., Erie, Pa., and Honolulu, Hawaii
Group IV:
Winning City: Schenectady, N. Y.
Honorable Mention Cities: Pasadena, Calif., Evanston, Ill., Madison, Wis., Charleston, S. C., and Harrisburg, Pa.
Group V:
Winning City: Hackensack, N. J.
Honorable Mention Cities: Santa Ana, Calif., Watertown, N. Y., Pittsfield, Mass., Maplewood, N. J., and Hagerstown, Md.
Group VI:
Winning City: Palo Alto, Calif.
Honorable Mention Cities: Chestertown, Md., Helena, Mont., Winnetka, Ill., McComb, Miss., Emporia, Kans., and Richmond, Ky.

1934 CITY HEALTH CONTEST AWARDS †

Special Award Cities (Those cities which have twice won First Award and in 1934 have maintained their previous high standards): Brookline, Mass., Detroit, Mich., Milwaukee, Wis., New Haven, Conn., and Syracuse, N. Y.
Group I:
Winning City: Baltimore, Md.
Honorable Mention City: Buffalo, N. Y.
Group II:
Winning City: Newark, N. J.
Honorable Mention Cities: Minneapolis, Minn., Toledo, Ohio, and Dallas, Tex.
Group III:
Winning City: Honolulu, Hawaii
Honorable Mention Cities: Hartford, Conn., Grand Rapids, Mich., Springfield, Mass., Duluth, Minn., Yonkers, N. Y., Reading, Pa., and Utica, N. Y.
Group IV:
Winning City: Pasadena, Calif.
Honorable Mention Cities: Kalamazoo, Mich., Schenectady, N. Y., Evanston, Ill., Waterbury, Conn., Sacramento, Calif., Binghamton, N. Y., San Jose, Calif.,

† Group I—Cities of over 500,000 population
" II—Cities of 250,000 to 500,000 population
" III—Cities of 100,000 to 250,000 population
" IV—Cities of 50,000 to 100,000 population
" V—Cities of 20,000 to 50,000 population
" VI—Cities under 20,000 population

New Rochelle, N. Y., and Greensboro, N. C.

Group V:

Winning City: Hackensack, N. J.

Honorable Mention Cities: Watertown, N. Y., Pittsfield, Mass., Auburn, N. Y., Greenwich, Conn., and Santa Barbara, Calif.

Group VI:

Winning City: Palo Alto, Calif.

Honorable Mention Cities: Englewood, N. J., Cliffside Park, N. J., Hibbing, Minn., and Miami Beach, Fla.

1934 RURAL HEALTH CONTEST AWARDS *

Northeastern Division:

Winning Counties: Cattaraugus County, N. Y., and Westchester County, N. Y. (tied)

Honorable Mention Counties: Columbia County, N. Y., Barry County, Mich., Southern Berkshire District, Mass., Allegan County, Mich., and Eaton County, Mich.

Eastern Division:

Winning County: Kent County, Md.

Honorable Mention Counties: Davidson County, Tenn., Ohio County, W. Va., Rutherford County, Tenn., Washington County, Md., Forsyth County, N. C., and Sullivan County, Tenn.

Southeastern Division:

Winning County: Glynn County, Ga.

Honorable Mention Counties: Charleston County, S. C., Lauderdale County, Miss., Pike County, Miss., and Leon County, Fla.

North Central Division:

Winning County: Woodbury County, Iowa

Honorable Mention County: Lyon County, Kan.

South Central Division:

Winning County: El Paso County, Tex.

Honorable Mention Counties: None

Western Division:

Winning County: San Joaquin Health District, Calif.

Honorable Mention Counties: Los Angeles County, Calif., Santa Barbara County, Calif., Spokane County, Wash., and Yakima County, Wash.

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* Northeastern Division: Connecticut, Illinois, Indiana, Maine, Massachusetts, Michigan, New Hampshire, New Jersey, New York, Ohio, Pennsylvania, Rhode Island, Vermont, and Wisconsin.

Eastern Division: Delaware, Kentucky, Maryland, North Carolina, Tennessee, Virginia, and West Virginia.

Southeastern Division: Alabama, Florida, Georgia, Mississippi, and South Carolina.

North Central Division: Colorado, Iowa, Kansas, Minnesota, Missouri, Montana, Nebraska, North Dakota, South Dakota, and Wyoming.

South Central Division: Arkansas, Louisiana, New Mexico, Oklahoma, and Texas.

Western Division: Arizona, California, Idaho, Nevada, Oregon, Utah, and Washington.

Studies and Surveys

Made by the Committee on Administrative Practice (1925–1935)

State	Surveyed City	Surveyed County	Surveyed State
Arkansas	(1925) Little Rock		
California	(1927) Burbank (1927) Glendale (1928) South Pasadena (1929) San Francisco*	(1928) Los Angeles (1929) Santa Barbara	
Canada	(1928) Montreal*		
Colorado	(1928) Denver*		
Connecticut	(1934) Hartford* (1935) Hartford (Indigent Sick Study)		
District of Columbia	(1929) Washington*†		
Georgia	(1927) Athens (1928, 1929, 1930 resurveyed)	(1927) Clarke (1928, 1929, 1930 resurveyed)	
Hawaii	(1929) Honolulu*		
Illinois	(1927) Chicago (1931) Chicago (1933) Chicago (1935) Chicago (Tb. Study)		
Indiana	(1926) Terre Haute		
Iowa	(1925) Des Moines (1935) Des Moines*		
Kansas	(1924) Kansas City†		
Kentucky	(1935) Louisville¶		
Maine	(1931) Bar Harbor		
Massachusetts	(1933) Cambridge (Tb. Study) (1930) Everett (1927) Hingham (1927) Quincy (1929 resurveyed) (1927) Lowell* (1929) Milton (1928) Wellesley	(1930) Southern Berkshire* (1931) Nashoba*	(1929) Mass.* (1935) Mass. (Laws)
Michigan	(1934) Detroit Clinic*	(1935) Mich. Community Health Project	(1928) Mich.*
Missouri	(1930) Kansas City*† (1927) St. Louis†		
Mississippi		(1931) Pike (1931) Lauderdale	
New Jersey	(1926) Elizabeth (1933) Montclair‡	(1928) Monmouth†	
New Mexico			(1934) N. M.*
New York	(1924) Syracuse (1927, 1928 resurveyed) (1934) Syracuse Study (Woodycrest San.) (1926) New York City (1932) New York City (1933) New York City (Harlem)	(1926) Cattaraugus (1927, 1928, 1929 resurveyed) (1928) Tompkins	

State	Surveyed City	Surveyed County	Surveyed State
New York (cont.)	(1934) New York City Appraisal* (1934) New York City Personnel Study (1927) Olean		
North Carolina	(1935) Charlotte	(1928) Pitt	
North Dakota	(1927) Fargo* (1928, 1930 resurveyed)		
Ohio	(1927) Cincinnati* (1926) Springfield*	(1926) Preble*	(1928) Ohio* (1931) Ohio*
Oregon	(1929) Portland*	(1927) Marion (1928, 1929, 1930 re-surveyed) (1929) Jackson	
Pennsylvania	(1924) Wilkes Barre (1927) Scranton†		
Rhode Island	(1928) Warwick* (1929) Providence*†		
South Carolina	(1927) Spartanburg*		
Tennessee	(1926) Knoxville* (1924) Memphis (1930 resurveyed) (1929) Nashville	(1930) Shelby* (1927) Rutherford (1928, 1929, 1930 re-surveyed) (1929) Davidson* (1930) Gibson (1930) Sullivan	
Texas	(1925) Austin (1925) Fort Worth		
Washington	(1934) Tacoma‖	(1935) Spokane*§	
West Virginia	(1924) Charleston		

* Survey printed
† Hospital also
‡ Hospital only
‖ City Contest free survey (1934)
¶ City Contest free survey (1935)
§ Rural Contest free survey (1935)

The American Museum of Hygiene

YOUR committee is particularly pleased to report the further crystallization of interest in Chicago. Although the local committee organization which was referred to in our report last year did not materialize, definite progress is being made under the leadership of Max Epstein. He has prepared, with the assistance of members of your committee, a preliminary descriptive outline for what is tentatively designated a Center of Health. This very well carries out the concept of the committee for a broad program embracing all phases of health and public health presented through the most modern exhibit and educational devices. This proposal has been favorably received by the Planning Committee of the Chicago Exposition Authority, which group has in hand the creation of a permanent exposition on part of the site of the Century of Progress.

We look forward to the first separate American Museum of Hygiene being a permanent and important unit in that exposition. Former plans for developing a health and medical section of the Museum of Science and Industry in Chicago, using exhibits from the Century of Progress as a nucleus, have been laid aside pending this new development. The desirability of a separate institution has continuously been stressed with interested people in Chicago.

A second development of importance is taking place in New York. In this city, work is already under way in constructing animated exhibits for a new section on hygiene in the American Museum of Natural History. This work is financed by a P.W.A. grant and members of your committee are advising with those responsible for this section. We have also made available to them the complete files of information which the committee has assembled. Although the present plans for this section fall short of the complete program which was proposed to the Museum of Natural History in 1930 by the secretary of your committee, it is a progressive step in health education. As such, it may serve to stimulate interest in the eventual establishment in New York City of a Museum of Hygiene adequate to the opportunities which this great city offers.

A third development of considerable interest has occurred on the West Coast. During the year the Western Branch of the American Public Health Association created a Committee on Health Education Exhibit Material. The studies of that committee have led it to conclude that the Western Branch may well become active and undertake a continuous program in this field. It believes particularly that "an unusual opportunity exists for the presentation of an outstanding exhibit or museum on public health at the International Exposition to be held in the San Francisco Bay area in 1938." A resolution was adopted by the Western Branch approving such an undertaking and including the proposal that such an exhibit be made the nucleus of a permanent public health museum to be housed in some city in the Western Branch area. Your Association committee has offered its

aid to the committee of the Western Branch and the two committees will coöperate in activities looking to the development of a Museum of Hygiene in the West.

Other manifestations of interest have appeared in Philadelphia and Buffalo. In the latter city the Museum of Science has created a "Hall of Man" which was opened in the spring of this year. The exhibit which was brought from the German Hygiene Museum by the Committee on Scientific Exhibits of the American Public Health Association and shown at our Pasadena Meeting has been placed in the Hall of Man for permanent display.

In conclusion, your committee feels that with returning normal economic conditions even further developments of the museum idea may be expected. The committee therefore recommends its continuation.

VICTOR G. HEISER, *Chairman*
HOMER N. CALVER, *Secretary*
BERTRAND BROWN
LOUIS I. DUBLIN
SALLY LUCAS JEAN
EVART G. ROUTZAHN
C.-E. A. WINSLOW

Current Health Department Practices*

THE Association is constantly receiving requests for information concerning the status and practices of local health departments. These inquiries most commonly relate to personnel and budget. In an effort to meet this demand, in part at least, the Sub-Committee on Current Practices of Health Departments has secured clerical assistance whereby the data coming to the office of the Association through the City and County Health Conservation Contests are recorded on forms displayed in Figures I, II, III and IV.

This information is made available to health officers and others who may request it. The names of the cities and counties together with the years for which data are available appear on the list which follows.

* Report on the work of the Sub-Committee of the Committee on Administrative Practice.

HEALTH JURISDICTIONS AND YEARS FOR WHICH DATA ON EXPENDITURES AND PERSONNEL HAVE BEEN COMPILED

(FIGURES I AND II)

x Cities for which information on expenditures and personnel is available through the card index file maintained by the Sub-committee on Current Health Department Practices in the Central Office.

State	*Jurisdiction*	*1930*	*1931*	*1932*	*1933*	*1934*
Arizona						
	Jerome		x			
	Phoenix					x
	Prescott	x	x			
	Yuma	x				
California						
	Alameda					x
	Fresno	x	x	x		
	Glendale				x	
	Lodi	x	x	x		
	Long Beach				x	
	Los Angeles			x	x	
	Oakland		x	x	x	
	Ontario	x	x			
	Palo Alto	x	x	x	x	
	Pasadena	x	x	x	x	x
	Pomona		x	x		x
	Redlands	x	x		x	
	Richmond	x	x	x		
	Riverside	x	x	x	x	x
	Sacramento	x	x	x	x	x
	San Francisco	x	x	x		
	San Jose					x
	Santa Barbara	x	x	x		x
	South San Francisco					x
	Torrance				x	

State	*Jurisdiction*	*1930*	*1931*	*1932*	*1933*	*1934*
Colorado						
	Denver	x	x	x		
	Pueblo	x	x		x	x
Connecticut						
	Greenwich	x	x		x	x
	Hartford	x		x	x	x
	Middletown			x		
	New Britain	x				
	New Haven	x	x	x	x	x
	Waterbury	x				x
	West Hartford		x	x		
Delaware						
	Wilmington	x	x		x	
Dist. of Columbia						
	Washington	x	x	x		
Florida						
	Bradenton	x	x			
	Miami Beach	x	x		x	
	Ocala	x	x	x		
	Tallahassee				x	
	Tampa	x	x	x	x	
Georgia						
	Albany	x	x			
	Athens	x	x			x
	Atlanta	x	x	x	x	x
	Augusta	x		x		x
	Brunswick				x	
	Macon	x	x	x		
	Rome	x	x			
Illinois						
	Aurora	x	x	x	x	
	Champaign	x				
	Chicago		x	x	x	
	Decatur	x	x	x	x	x
	Evanston	x	x	x		x
	LaSalle					x
	Peoria	x	x	x		
	Peru			x	x	
	Rockford					x
	Winnetka	x	x	x	x	
Indiana						
	Evansville				x	x
	LaPorte		x		x	
	Mishawaka		x	x		
Iowa						
	Boise	x	x	x		
	Burlington	x	x			
	Des Moines	x	x	x		x
	Dubuque	x	x			
	Fort Dodge		x			
	Washington					x
Kansas						
	Abilene	x	x			
	Arkansas City	x				
	Atchison		x	x		
	El Dorado	x	x			
	Emporia	x	x	x	x	
	Eureka	x				

State	*Jurisdiction*	*1930*	*1931*	*1932*	*1933*	*1934*
Kansas (Cont.)						
	Hutchinson	x	x			
	Junction City	x	x			
	Kansas City	x	x			
	Manhattan	x	x	x	x	
	Topeka	x	x	x	x	x
	Wellington	x				
	Winfield	x	x	x		x
Kentucky						
	Louisville		x	x	x	x
	Owensboro	x	x	x		
	Pikeville			x		
	Richmond				x	
Louisiana						
	Natchitoches	x				
	New Orleans	x				
	Shreveport	x				x
Maine						
	Portland		x	x		
Maryland						
	Baltimore			x	x	x
	Chestertown	x	x	x	x	
	Cumberland	x	x			
	Elkton	x				
	Hagerstown				x	
Massachusetts						
	Attleboro	x	x			
	Brookline	x	x	x	x	x
	Framingham	x	x			
	Malden				x	
	Pittsfield	x	x	x	x	x
	Springfield	x	x	x		
Michigan						
	Battle Creek	x	x			
	Benton Harbor	x	x			
	Detroit	x	x	x	x	x
	Flint	x	x	x	x	
	Grand Haven	x				
	Grand Rapids	x	x	x	x	x
	Kalamazoo		x	x	x	x
	Muskegon	x	x			
	Muskegon Heights	x	x			
	Pontiac	x	x			
	Saginaw	x	x			
	South Haven	x	x	x		
Minnesota						
	Albert Lea				x	
	Duluth	x	x	x	x	x
	Eveleth					x
	Eureka		x	x		
	Faribault		x			x
	Hibbing					x
	Minneapolis	x	x	x	x	x
	St. Paul	x	x			
	Virginia				x	
	Winona	x	x	x		
Mississippi						
	Greenville	x	x	x	x	
	Natchez	x				

FIGURE I. CARD (FRONT) USED FOR RECORDING DATA CONCERNING EXPENDITURES AND PERSONNEL OF CITY HEALTH DEPARTMENTS

Name of Health Jurisdiction

Address: State City Street

Name of Health Officer

Population Served by H.D. (19) (19) (19) (19)

EXPENDITURES FOR FIELD SERVICE	19			19			19			19		
	Expenditure	Per Cap	% of Total	Expenditure	Per Cap	% of Total	Expenditure	Per Cap	% of Total	Expenditure	Per Cap	% of Total
Administration												
Vital Statistics												
General Sanitation												
Food, Drugs, Milk												
Child Health Supervision												
Adult Health Supervision												
Communicable Disease Control												
Tuberculosis												
Venereal Diseases												
Laboratory												
Health Education												
Public Health Nursing												
Sub Total												
Other Field Services (specify)												
Sub-total												
Grand Total Field Services												

FIGURE II. CARD (BACK) USED FOR RECORDING DATA CONCERNING EXPENDITURES AND PERSONNEL OF CITY HEALTH DEPARTMENTS

EXPENDITURES FOR INSTITUTIONS AND CLINICAL SERVICES	19			19			19			19		
	Expenditure	Per Cap	% of Total	Expenditure	Per Cap	% of Total	Expenditure	Per Cap	% of Total	Expenditure	Per Cap	% of Total
Tuberculosis Hospitals												
Communicable Disease Hospitals												
General Hospitals												
Other Institutions & Clinical Serv. (specify)												
Total Institutions												
Grand Total—Field, Instit'l & Clin. Serv.												

PERSONNEL (Salaried Employees in Field Service)

PERSONNEL CLASSIFICATION	19				19				19				19			
	F. T.	Salary Range	P. T.	Total	F. T.	Salary Range	P. T.	Total	F. T.	Salary Range	P. T.	Total	F. T.	Salary Range	P. T.	Total
Physicians																
Veterinarians																
Engineers																
Sanitary Inspectors																
Nurses																
Laboratory (Technical)																
Clerks (All types)																
Other Employees																
Total																

State	*Jurisdiction*	*1930*	*1931*	*1932*	*1933*	*1934*
Missouri						
	Carrollton	x	x			
	Columbia				x	
	Hannibal	x	x	x		
	Independence	x	x			
	Joplin		x	x		
	Kansas City	x	x	x	x	
	St. Louis	x	x		x	
Montana						
	Billings		x	x		
	Bozeman		x	x		
	Helena		x	x	x	x
	Great Falls		x	x	x	
Nebraska						
	Lincoln	x	x	x	x	x
	Omaha	x	x	x	x	
New Hampshire						
	Berlin					x
New Jersey						
	Cliffside Park					x
	East Orange	x	x	x	x	
	Englewood				x	x
	Hackensack					x
	Maplewood	x	x		x	
	Newark	x	x	x	x	x
	Orange	x	x		x	
	Ridgewood	x	x			
	South Orange	x				
	West Orange	x	x	x	x	
New Mexico						
	Carlsbad	x	x			
	Roswell			x		
Nevada						
	Los Vegas				x	
New York						
	Albany		x	x	x	x
	Amsterdam	x			x	x
	Auburn	x	x	x		x
	Binghamton	x	x	x		x
	Buffalo	x	x	x	x	x
	Ithaca	x	x			
	Middletown	x	x	x		
	Newark					x
	Newburgh	x	x	x		
	New Rochelle					x
	Ogdensburg	x	x	x	x	
	Owego	x				
	Plattsburg	x	x			
	Queens	x		x	x	x
	Rochester	x	x	x	x	
	Schenectady	x	x	x	x	x
	Syracuse	x	x	x	x	x
	Utica	x	x	x	x	x
	Watertown	x	x	x	x	x
	White Plains	x				
	Yonkers	x	x	x	x	

State	*Jurisdiction*	*1930*	*1931*	*1932*	*1933*	*1934*
North Carolina						
	Durham	x				
	Greensboro	x	x	x	x	x
	Raleigh	x	x			
	Rocky Mount				x	
	Winston Salem	x				
North Dakota						
	Fargo	x	x			
Ohio						
	Akron	x	x	x	x	x
	Cincinnati	x	x	x	x	
	Dayton	x				
	Delaware	x	x			
	Elyria				x	
	Hamilton	x	x			
	Lakewood	x	x	x		x
	Lima			x		
	Marietta					x
	Marion	x	x			
	Middletown	x	x	x		
	Sandusky	x	x			
	Sidney	x				
	Springfield	x	x			
	Steubenville					x
	Toledo	x	x	x	x	x
Oklahoma						
	Blackwell					x
	Woodward	x				
Oregon						
	Medford	x				
	Portland	x	x	x		
	Salem	x	x			
Pennsylvania						
	Erie	x	x	x	x	x
	Harrisburg	x	x			
	Philadelphia	x	x			
	Pittsburgh	x	x	x	x	x
	Reading	x	x	x	x	x
	York					x
Rhode Island						
	Newport	x	x	x	x	
South Carolina						
	Charleston	x	x	x	x	
South Dakota						
	Huron	x	x			
	Mitchell	x				
Tennessee						
	Chattanooga	x	x		x	
	Clarksville	x	x			
	Johnson City					x
	Knoxville	x				
	Memphis	x	x			
	Nashville	x	x	x	x	x
Texas						
	Amarillo					x
	Austin	x	x		x	
	Beaumont	x	x	x		

State	*Jurisdiction*	*1930*	*1931*	*1932*	*1933*	*1934*
Texas (Cont.)	Corsicana	x				
	Dallas	x	x	x	x	x
	El Paso	x	x	x		
	Fort Worth	x	x	x	x	x
	Houston	x	x	x	x	x
	Jacksonville	x				
	Palestine	x				
	San Antonio	x	x			
	Sherman		x	x		
	Texarkana	x				
	Tyler					x
Utah						
	Logan					x
	Provo					x
	Salt Lake City	x	x	x	x	
Vermont						
	Springfield	x	x	x		
Virginia						
	Charlottesville		x			
	Fredericksburg	x	x			
	Norfolk	x	x			
	Petersburg	x				
	Roanoke	x	x	x	x	x
Washington						
	Bellingham			x	x	
	Spokane	x	x	x		
	Tacoma	x		x	x	x
	Walla Walla	x	x	x	x	
West Virginia						
	Beckley			x	x	
	Bluefield	x	x	x	x	x
	Charleston	x	x	x	x	x
	Princeton		x	x		
Wisconsin						
	Eau Claire		x			
	Kenosha	x	x			
	Milwaukee	x	x	x	x	x
	Oshkosh	x	x			
	Sheboygan	x	x	x		
	Shorewood	x	x			
	Wausau	x				

COUNTY HEALTH JURISDICTIONS FOR WHICH DATA ON EXPENDITURES AND PERSONNEL HAVE BEEN COMPILED FOR THE YEAR 1934

(FIGURES III AND IV)

State	*Jurisdiction*	*State*	*Jurisdiction*
Alabama	Calhoun	California	Contra Costa
	Etowah		Los Angeles
	Morgan		Santa Barbara
	Tuscaloosa		San Bernardino
Arizona	Gila		San Joaquin
	Maricopa		Stanislaus
Arkansas	Monroe		

FIGURE III—CARD (FRONT) USED FOR RECORDING DATA CONCERNING EXPENDITURES AND PERSONNEL OF COUNTY HEALTH DEPARTMENTS

Name of Rural Health Unit or District:
Address: Street City State
Health Officer:
Population: (193) (193) (193) (193)
Remarks:

	193					193				
	Number of Employees		Expenditures			Number of Employees		Expenditures		
	Full-Time	Part-Time	Amount	Per Cap.	% of Total	Full-Time	Part-Time	Amount	Per Cap.	% of Total
Health Officer										
Medical Personnel										
Nurses										
Sanitation Officers										
Other Personnel (Classify)										
Operating Expenses (Classify)										
Total										

Source of Support	193		193	
County				
State				
Federal				
Non-Official (List)				
Total				

FIGURE IV—CARD (BACK) USED FOR RECORDING DATA CONCERNING EXPENDITURES AND PERSONNEL OF COUNTY HEALTH DEPARTMENTS

	193					193				
	Number of Employees		Expenditures			Number of Employees		Expenditures		
	Full-Time	Part-Time	Amount	Per Cap.	% of Total	Full-Time	Part-Time	Amount	Per Cap.	% of Total
Health Officer										
Medical Personnel										
Nurses										
Sanitation Officers										
Other Personnel (Classify)										
Operating Expenses (Classify)										
Total										

Source of Support	193		193	
County				
State				
Federal				
Non-Official (List)				
Total				

State	*Jurisdiction*
Delaware	Kent
	Newcastle
	Sussex
Florida	Leon
Georgia	Glynn
	Jenkins
Iowa	Woodbury
Kansas	Lyon
Kentucky	Barren
	Fayette
	Jefferson
Louisiana	Caddo Parish
	DeSoto Parish
	Rapides Parish
Maryland	Kent
	Montgomery
	Washington
	Wicomico
Massachusetts	Southern Berkshire
Michigan	Allegan
	Barry
	Eaton
Minnesota	St. Louis
Mississippi	Lauderdale
	Leflore
	Lee
	Pike
Missouri	Buchanan
	Greene
New Mexico	Bernalillo
	Eddy
New York	Cattaraugus
	Columbia
	Westchester
North Carolina	Forsyth
	Moore
Ohio	Darke
	Delaware
	Lorain
Oregon	Jackson
South Carolina	Charleston
Tennessee	Davidson
	Hamilton
	Rutherford
	Shelby
	Sullivan
	Washington
Texas	El Paso
Virginia	Fairfax
Washington	King
	Spokane
	Walla Walla
	Yakima
West Virginia	Ohio
	Raleigh

Several very interesting analyses of the data have already been made and published in the *American Journal of Public Health.*

March Journal—A Central Information Service on Current Practices of Health Departments, by Joseph W. Mountin, M.D., presents the purposes of the Sub-Committee and summary tables on total and per capita expenditures in certain cities for health service during the 4 year period, 1930–1933.

May Journal—Specific Expenditures and Personnel of Official Health Agencies in Certain Cities, by Joseph W. Mountin, M.D., presents an analysis of per capita expenditures for the year 1933 by specific functions and an analysis of number of health department personnel employed according to professional classification.

July Journal—Analysis of Public Health Expenditures by Geographic Sub-Divisions, by W. F. Walker, Dr.P.H., presents total and per capita expenditures in certain cities by geographic sub-divisions of the United States. Also brings up to date 1934 (data added) table on total and per capita expenditures by population groups published in March issue of *Journal.*

October Journal—Public Health Expenditures in Selected Cities by Nonofficial Agencies, by James Wallace, M.D., and Louis Feldman, is an analysis of expenditures by nonofficial agencies reported in connection with the City Health Conservation Contests for the 5 year period, 1930–1934.

An effort is now being made to complete the information for the missing years and to expand the list to areas not entered in the Health Conservation Contests. The coöperation of the health officers in this undertaking is essential to its success.

JOSEPH W. MOUNTIN, *Chairman*

Refrigeration of Foods and Its Relation to Present-Day Public Health*

IN accordance with the suggestion of the previous Committee on Foods, your present committee has endeavored to trace recent developments in certain of the food industries with the purpose of evaluating these changes from the standpoint of their relationship to public health. The particular field chosen for our report is that of food refrigeration, a topic hardly capable of complete discussion in the limited time available, but worthy of attention because of its intimate association with the health and well-being of everyone.

Since the invention of the first compression refrigerating machine about 1834, the possibilities of refrigeration have become increasingly apparent, although wide usage of refrigeration facilities has been attained only within the past generation. Today we find refrigeration of paramount importance and a necessity from the standpoint of economics, health, and convenience. The diet of mankind has been materially improved both in variety and quality by refrigeration which enables the year-round consumption of foods from even the most distant countries at prices within reach of the great majority, despite seasonal harvests and long transportation.

Our country is more fortunate in respect to our major food supplies than are some others, such as Great Britain, which draws largely from overseas, but with the increase in uses for refrigeration an industry of great magnitude has developed in the United States to meet augmented needs of this nature. Sir Frank Smith, Secretary of the Department of Scientific and Industrial Research of Great Britain stated last September that the refrigerated space regularly required to transport England's food supplies was equivalent to a floating cold store 20 ft. high, 50 ft. wide and 20 miles long, and that the public cold storage on land amounted in addition to approximately half that content.

In comparison, using statistics presented recently by the American Institute of Refrigeration, the United States in 1931 had refrigerated warehouses which together would approximate a similar chamber 140 miles long, our 119,000 refrigerated freight cars adding 45.5 miles, our ships a further 22 miles, and the domestic mechanical refrigerators sold in the past 5 years another 4.8 miles. Thus we have as a nation a potential refrigerator for foods 50 ft. x 20 ft. over 200 miles in length (or greater than the distance from New York to Baltimore) exclusive of ice boxes in the homes, stores, and restaurants, and the 42 million tons of artificial ice produced during the same year in addition to tremendous quantities of natural ice.

In January, 1935, according to the U. S. Department of Agriculture, there were in cold storage:

Butter	23,587	tons
Cheese	51,098	"
Frozen eggs	32,439	"
Poultry	61,000	"
Meats	479,548	"
Fruits	31,384	"
Fish	34,672	"
Apples	8,890,000	barrels

* Report of the Committee on Foods.

During 1934 we exported 97,715 tons of refrigerated meats and over 544,000 tons of vegetables and fruits.

In 1912 a symposium was held by this Association during which the various aspects of cold storage relating to public health were discussed. At that time refrigeration, or cold storage of foods as it was then termed, was looked on with some suspicion by the general public. In this symposium the late Professor Sedgwick made the following statement:

> In short, cold storage is one of the greatest boons mankind has ever known. From the sanitary as well as the economic point of view it is a blessing because it gives us a much greater variety of health-giving food than existed in the older days.

The passage of over 2 decades has amply verified this statement because there has been a great increase in the quantity of foods subjected to refrigeration, the number of products, and the extent to which each is so handled. The necessity for *continuous* refrigeration from the time of harvest or slaughter until consumption is becoming more clearly recognized. The precooling of products before shipment is now commonly practised by many shippers of fruits, vegetables, and other foods, who formerly felt it unnecessary. Careful attention in these respects has doubtless reduced spoilage which is a large factor in our more perishable food supplies.

Today, as was always the case, the refrigeration of foods should be considered as one of the very best means of food preservation, provided it is carried out under proper conditions. Refrigeration never has and never will make good foods out of poor ones, but, fortunately, this fact has become thoroughly understood. It is also evident that the public no longer looks askance at refrigerated foods, because otherwise no such expansion of this type of food handling as has taken place would have been possible.

The "cold storage" label required by law on foods subjected to refrigeration for any length of time has ceased to cast the stigma which in earlier years it sometimes attached to foods preserved in this manner.

Food refrigeration still needs and deserves proper supervision. Only foods of sound quality should be worthy of refrigeration, and such storage should be controlled as to duration, temperature, humidity, and other physical conditions. The various state laws concerning foods and food storage are effective only within their respective borders, while the inspection of all foods entering interstate commerce is vested in the Food and Drug Administration of the U. S. Department of Agriculture, which enforces the federal food and drug acts. The inspection of meats and supervision of packinghouse products and cold storage of the same is under the jurisdiction of the Bureau of Animal Industry, and its regulations also apply to all products of this nature which cross state borders.

The quality and edibility of foods, and refrigerated foods as well, is dependent more upon their physiological or biochemical age, if such a term may be used, than on their chronological age. The physiological or biochemical age is dependent on the extent to which the processes of maturation in living materials—such as fruits and vegetables; and the catabolic enzymes contained in dead materials, such as animal products; plus the activity due to the bacteria, molds and yeasts they contain—have progressed, and the changes which have resulted therefrom. In other words, taking eggs as an example, an egg laid only a few days and improperly stored may be of inferior quality to another egg laid the same day but carefully and immediately cooled and maintained under optimum

conditions of refrigerated storage for several months. As time goes on and more is learned about such physiological and biochemical changes, it would seem that the more logical method for the determination of quality and edibility of cold storage products would be the evaluation of such changes, together with the direction of more attention to the preparation of such products for storage, and their actual storage conditions, rather than the emphasizing of storage time, or chronological age, which may sometimes be a fallacy. At present the different state laws concerning cold storage show a considerable divergence in respect to the time limits during which foods may undergo refrigerated storage.

Microbiological studies made on refrigerated foods indicate that not all microörganisms are equally susceptible to the effects of low temperature. Different foods provide media of widely varying chemical composition which influence the reproduction of microörganisms to a marked degree, as do also the temperature and the duration of refrigeration. Certain cold-tolerant types of microörganisms persist in spite of the most rigorous treatment. The usual refrigeration temperatures cut down the rate of reproduction and tend to reduce bacterial counts. At the lower temperatures this effect is more marked, but refrigeration should not be considered a means of sterilization. Refrigeration cannot be expected to be lethal to all food poisoning organisms, or to inactivate bacterial toxins; but it should retard the growth of pathogens and the production of toxins. The proper selection and preparation of foods for refrigeration, sanitary handling of the same, and constant refrigeration until they are used, should minimize hazards.

The sanitation of refrigeration facilities has been improved in recent years. The industry itself has voluntarily made many improvements which have raised standards to a higher level, although due credit must be given to those governmental agencies which have so capably supervised and encouraged such procedures.

Much attention has been paid of late to a study of the best conditions of storage for specific foods, particularly fruits, vegetables, and meats, and it has been found that optimum conditions of temperature and humidity differ to a considerable extent. The storage of foods in the presence of controlled atmospheres containing gases, especially carbon dioxide, has been found desirable in respect to fruits and meats and has recently come into use in English warehouses and ships.

In addition to refrigerated storage and transportation, which may be at temperatures above or slightly below the freezing point, there has been a vast increase in the freezing of foodstuffs. The last decade has seen the birth of a new industry which has already made its impression on the diet of many of our people, namely, that of quick-freezing as distinguished from the older methods.

Another closely allied innovation is the development of quick-frozen methods by which the consumer units of relatively small size can be frozen within the package. This processing of quick-frozen foods, which was first applied to fish, has been extended to many other food products, including practically all meats, shellfish, vegetables, fruits, and even corn on the cob. Certain of the vegetable products are subjected to a short blanching process or heat treatment previous to freezing, in order to inhibit enzyme action with its concomitant changes in color and flavor, and facilitate packing operations. These products even after storage for a number of months at refrigeration temperatures (usually 0° F. or lower) compare very closely in

flavor and taste with the fresh, thus products can be packed at their optimum condition of maturity and preserved in substantially that condition until consumed. This is not the case with many fruits and some vegetables, not frozen, subjected merely to storage at reduced temperatures in transit, as such products are often picked before full maturity.

It must be emphasized that frozen foods, like all other refrigerated foods, require *continuous* low temperatures to minimize the growth of microörganisms and the biochemical activity of enzymes. Unless low temperatures are maintained until the products are about to be used, spoilage will occur and health hazards may result. This is well known in respect to unrefrigerated foods, which may be kept only for limited periods, but it applies equally to those which have been refrigerated or frozen.

Quick-frozen products are now available in many sections of the country where refrigeration facilities at the lower temperatures given are available. The maintenance of such low temperatures for storage warehouses in many rural districts, in retail stores and homes, has been unusual in the past but appears to be on the increase due to this development. In the larger cities such facilities have become quite common.

Numerous workers have been concerned in recent years with the nutritive aspects of foods, particularly from the standpoint of vitamins, and certain of these researches have related to refrigerated foods in storage. More attention in this respect has been paid to fruits and vegetable products and their vitamin C content, because certain of them have attained marked popularity and increased consumption due in part to such content. Such evidence as is at hand indicates that refrigeration of foods containing vitamin C tends to conserve such content in comparison with similar products not refrigerated during storage, although periods of months may diminish the concentration in some foods unless very low temperatures are employed. No evidence that the other known vitamins are deleteriously affected by the use of low temperature has been presented. This applies also to frozen foods.

It is believed that during the past few years of reduced incomes the economies of refrigeration have enabled many families to obtain foods in the fresh form during off-seasons at a cost which might otherwise have been prohibitive, thus allowing better balanced and more complete diets which contribute to the health of the individual.

An example of economy due to refrigeration is the reported distribution to families on welfare of frozen meat produced by the emergency slaughter of food animals in the drought areas by government agencies. These animals, doomed to die of malnutrition otherwise, were conserved or salvaged. It is hoped that such products, helpful as they may be as a source of food, will not be considered by the consumers as representative of the high quality usually found in refrigerated products.

The applications of refrigeration in the food industries have increased markedly in the post-war years. Omitting the dairy industries, which are more properly the field of another committee, it is worthy of mention that refrigeration is now used extensively in bakeries, breweries, candy and chewing gum factories, canneries, food oil hydrogenation plants, cocoa manufacturers, gelatin factories, packing houses, yeast plants, and many others. In some cases the use of low temperatures is for physical reasons, but in large part it is for the conservation of prod-

ucts against spoilage and the possibilities of injury to the health of the consumer. Refrigeration has also been shown to be lethal for various insect pests including the European corn borer. It has also been found effective in killing the larvae of Trichinella spiralis which are the causative agents of trichinosis due to eating pork and its products.

Thus we may visualize refrigeration as one of the protective forces of our community which functions in large part unseen, although those who are observant may note warehouses at our docks and terminals, pre-coolers in the harvest regions, insulated and refrigerated freight cars and fast motor trucks—even tank trucks—display cases in our stores which have vastly improved the retail handling of perishable foods, and the vast army of domestic refrigerators in restaurants, hotels and homes which are of equal importance at the consumers end. More than 5,000,000 mechanical guardians of the home which maintain a constant low temperature have been sold in this country since 1930, giving some evidence of the reliance which our nation places on refrigeration. The use of such facilities will doubtless avoid many unfortunate cases of disease formerly caused by improperly handled foods.

There are evidences that such facilities might well be extended to include the refrigeration of some products not commonly refrigerated at present. Numerous outbreaks of food poisoning have been traced to cream-filled bakery products, some of which have caused deaths. Refrigeration is no substitute for cleanliness or proper materials and procedures in the manufacture of filled bakery products; *but it is recommended as a desirable type of storage for such ingredients as may not be heat treated in manufacture,* in transit, in the food shop, and last, but not least, in the home.

In summation, your committee views refrigeration as a faithful ally and servant to all food consumers, one which has become so intimately connected with our daily life and existence as to be practically indispensable. Like all servants, it requires proper and strict supervision. Fortunately, in this country, such supervision has been both adequate and capable with rare exceptions. The use and advantages of refrigeration bid fair to expand as time goes on, to the benefit of the health, comfort, and pocketbook of our nation.

Your committee recommends the continuation of the work of the Committee on Foods for another year.

B. E. PROCTOR, *Chairman*
H. C. BECKER
P. J. ZENTAY
J. H. TOULOUSE
C. S. LADD
H. N. PARKER
D. K. TRESSLER

Standard Methods for Microbiological Examination of Foods

PROGRESS toward the standardization and simplification of the microbiological analyses of food products has been made along 3 lines, namely, for fermented foods, for meats and meat products, and for shellfish. In accordance with the policy of this committee, bacteriologists experienced in the analysis of these types of foods have been requested to assume the leadership in the development of methods for each product.

Dr. C. S. Pederson, who has long been interested in fermented foods, agreed to promote methods for sauerkraut, pickles and other fermented products. Inasmuch as there is now very little information available on the types of microörganisms present in fermented foods, following the report by Dr. Pederson presented at the American Public Health Association Annual Meeting in Indianapolis in 1933, it seemed advisable to arrange for a series of examinations in various laboratories of both known and unknown samples of fermented foods.

Accordingly, Dr. Pederson prepared an outline of the types of tests he considered important. This outline was submitted to all members of the committee and others who were in a position to make helpful suggestions. The comments of each reviewer have been carefully considered and every suggestion incorporated. With a brief introduction and with some sections expanded to greater detail, this outline will serve as the basis for the examination of test samples soon to be submitted to some 6 or 8 different laboratories.

These preliminary analyses will be made in order to secure information as to the microflora generally found in fermented foods. From such information the methods, culture media, etc., best suited to the needs can be selected. Such general examinations can serve also to acquaint the analysts with fermented foods, as well as with the methods of analysis.

The development of methods for the examination of meats and meat products has received an unexpected and gratifying stimulation. The Bacteriological Sub-committee of the Technical Meat Committee of the National Canners Association has been studying this problem for some time and has joined its forces with the American Public Health Association in order that the greatest progress can be made with the limited personnel available. This is encouraging indeed and it is hoped real progress can be made along this line during the coming year.

In the field of shellfish examinations the committee has been ably represented by Dr. A. C. Hunter, who some time ago was made referee for developing standard methods for the examination of all types of marine products. The Association has been very active in work on oysters, and Dr. Hunter has taken an active part in the conference and developmental work.

At this convention, the results of many thousands of laboratory examina-

* Report of the Committee on Microbiological Methods of Food Examination of the Food and Nutrition Section.

tions on oysters, as well as the results of conferences and questionnaires on methods have been presented. Among these was a discussion by Dr. Hunter of the shellfish report from the control viewpoint.

Many different types of culture media have been used by various bacteriologists for the cultivation of anaerobic bacteria. Beef muscle extracts, beef heart extracts, liver infusions, chicken broths, vegetable broths, etc., all including a small portion of the original plant or animal tissue, have been prepared, used and recommended.

Anaerobic bacteria play an important part in food preservation and food spoilage, so this committee is vitally interested in any and all progress leading toward a more simplified practice in microbiological technic.

An extensive study of the value of different types of culture media for the cultivation of anaerobic bacteria has has been carried out by Dr. L. S. McClung. He was particularly interested in thermophilic anaerobes, but his survey of various media and methods of producing anaerobic conditions briefly summarizes the information to date, so that his selection of a method which is generally satisfactory for the cultivation of anaerobic bacteria is significant. In his study, the sealing of culture tubes with agar proved the most satisfactory. It is simple, rapid, and does not make the tubes difficult to clean, which is a serious problem in large laboratories.

Coöperation in this committee's work has been enthusiastic and it is hoped real progress can be made during the coming year.

LAWRENCE H. JAMES, *Chairman*
P. K. BATES
L. A. BRADLEY
E. J. CAMERON
E. P. GRIFFITHS
A. C. HUNTER
C. S. PEDERSON
F. W. TANNER
L. B. JENSEN

Milk and Dairy Products

DURING the past 3 years the Committee on Milk and Dairy Products has endeavored to review the scientific literature on vitamin D milks and to discuss certain aspects of their production which are of direct concern to administrative health officials.

As a result of reported clinical studies on vitamin D milks, a large number of cities have permitted the sale of these products. In some instances, these vitamin D milks are subject to control by means of ordinances or regulations, but in other instances, the sale of these products is not regulated by specific legal requirements.

In previous reports, the committee has attempted to show the status of these milks under existing laws defining milk and milk products. To illustrate the confusion on this subject which still prevails, the U. S. Public Health Service Milk Ordinance, which has been adopted in about 600 communities, merely states that vitamin D milks shall be produced in accordance with standards and regulations prescribed by the health officer, which obviously means that there is and will be an unfortunate lack of uniformity in control of these important products.

In contrast to this may be cited the regulations adopted during the past year by the Board of Health of New York City, which require, among other things, that the source of vitamin D and the number of units per quart shall be stated upon the labels. On the other hand, Dr. Paul B. Brooks, Deputy Health Commissioner of New York State, has expressed the opinion [1] that "no statement as to the number of units per quart shall appear on the cap or label." Since all market milk sold in this state, outside of New York City, is subject to the regulations of the state, this viewpoint, if incorporated in official control standards, would set up conflicting requirements within the same state.

With regard to the labeling of vitamin D milks, there appears to be a divergence of scientific opinion as to the use of comparisons between the potencies of vitamin D, as expressed in units for the several milks, and their equivalents in cod liver oil. This subject deserves careful consideration, with due regard to the results of clinical investigations.

The present mode of commercial advertising of the various vitamin D milks often tends to confuse the consuming public, especially when improper emphasis is given to units or to the question whether a certain milk is produced by "natural" or "artificial" means, when the clinical evidence indicates that all of these milks when properly produced are antirachitic.

Uniformity of administrative control of vitamin D milks of all types is essential. A noteworthy step toward the achievement of uniform standards and requirements for these products has been the establishment of the Vitamin Section of the Drug Division of the Food and Drug Administration of the federal government. In view of the facilities available to this federal agency, it is to be hoped that effective and reasonable standards for vitaminized products may be promulgated. This agency is in charge of Dr. E. M. Nelson, who is the Chairman of the

Committee on Standard Methods of Vitamin D Bio-assay of Milk, of the Food and Nutrition Section of this Association.

In order to standardize more effectively the physical and mechanical aspects of irradiation as applied to milk and to define the product, a conference of technical experts was convened in July in New York City. This meeting was called at the suggestion of the chairman of your committee, in his capacity as a public health official, and held under the auspices of the Wisconsin Alumni Research Foundation. As a result of this significant conference, a permanent committee of physicists, research engineers, and biochemists was appointed to define irradiation, and to formulate specifications for the process. The committee has already prepared a preliminary report. This action is to be commended as a progressive accomplishment looking toward the more effective correlation of a widespread practice of great significance to control officials and to the public health.

The work of this committee should lead to the establishment of reliable irradiation technic and the equipment of machinery with suitable indicating and recording devices which will make possible the official enactment of satisfactory requirements, and will permit of effective supervision by routine inspection. These procedures are analogous to present standards for and inspection of the process of pasteurization.

PASTEURIZATION OF CERTIFIED MILK

The noteworthy application of a scientifically proven safeguard to public health was the approval during the past year of the permissive pasteurization of certified milk, unanimously voted by the American Association of Medical Milk Commissions at their annual meeting in Atlantic City in June, 1935.

Section 58 of the Methods and Standards for Certified Milk has been amended to read as follows:

> Certified milk must be produced strictly in accordance with the Methods and Standards, and when so produced it may be subsequently pasteurized, provided it is labelled on the bottle cap with the words "Certified Milk—Pasteurized" in addition to the other requirements for the capping and sealing of bottles as described in Section 44.

A new section (No. 59) says:

> Pasteurization of Certified Milk must be done on the farm where it is produced and under the existing state and local rules and regulations. Equipment used for the pasteurization of Certified Milk shall not be used for the pasteurization of any other grade of milk.

This important action has been highly commended in editorials in the *American Journal of Public Health* for August, 1935, and in the *Journal of the American Medical Association* for August 24, 1935. Two pertinent quotations from these significant editorials are of special interest.

In discussing the action at Atlantic City, the *American Journal of Public Health* states:

> It was pointed out at that time, and we agree, that the action taken in favor of permissive pasteurization was a landmark in the development of certified milk, an episode which is as significant to dairy science and to public health as was the institution of certified milk itself in 1893. Physicians and consumers who desire a clean and safe raw milk can still obtain it, but an even greater proportion of discriminating physicians and consumers who prefer that the best milk obtainable be pasteurized will at last be satisfied. Health officials will now have added reasons for recommending and advocating increased consumption of these certified milks.

The *Journal of the American Medical Association* states:

> Now that the producers of certified milk and the medical milk commissions have adopted permissive pasteurization there should be benefit to all interested groups. Physicians desiring the highest quality of pasteurized milk for their patients will be able

to prescribe pasteurized-certified milk; producers of certified milk will be able to offer their customers a pasteurized product; public health authorities will be aided in their program of universal pasteurization, and milk consumers will have a wider choice among desirable milk supplies.

Certified Milk—Pasteurized is already on the market in Boston, Cincinnati, Detroit, Miami, and Philadelphia, and plans are under way to distribute it in New York and other large cities after October 15, 1935.

Health officials should endeavor to facilitate the distribution and sale of certified milk—pasteurized, and where necessary should adopt new legislation or amend existing laws to conform to the present methods and standards for certified milk. This has already been done in a number of states and cities. Methods of pasteurization should be those already sanctioned by law or set forth in the U. S. Public Health Service Milk Ordinance.

COMPOSITION OF MILK

The composition of certified milk must conform to rigid standards, based upon the natural characteristics of the dairy animals and influenced by scientific control of the constituents of ration of the cow. In some instances standardization of certified milk was practised, but the system has been discontinued as inadvisable.

Competition for commercial supremacy in the sales of market milks has in recent years often been based on "deeper cream line." Frequently this has led to standardization, by removal of skim milk by the dairyman by several methods, which practices may and do lead to contamination and even adulteration. For these reasons, such methods should not be tolerated. Obviously, if certified milk, which is intended primarily for infant feeding, should not be subject to such manipulation, other grades of milk, which are even more extensively employed as such an important food, should not be altered in composition on dairy farms where chances of serious contamination are likely to prevail.

In a study of standardization of milk on the farm by Button and Perry,[2] the conclusion is reached that practices of siphoning and separating involve unsanitary features; that fore-milking or the removal of skim milk by siphoning or gravity is not economical due to the loss of butter fat; and that the daily variations in herd tests make it difficult for dairymen to standardize their products so that they will contain the desired butter fat content. Unsanitary methods and conditions and financial losses to dairymen are both directly and indirectly of public health concern. In the first instance the product is subjected to bacterial contamination, and in the second case the dairyman is handicapped in his ability to maintain proper methods and equipment.

Perusal of existing laws defining milk reveals that standardization of milk on the dairy farm is generally illegal.

MISINFORMATION ON MILK

Much misinformation on milk and milk products is distributed by various self-appointed consumer organizations. Bulletins and other publications of these groups frequently contain erroneous and prejudiced facts, which are not only unreliable for consumers and unfair to the industry, but reflect upon the skill and integrity of health officials. Such distorted and irresponsible material should be condemned.

CONCLUSION

This report emphasizes the progress which is being made in the official establishment of uniform, reasonable, and intelligent control of various important milk products; point out certain ill-advised practices in milk pro-

duction; and the necessity for the enforcement of existing laws.

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Food Fallacies and Nutritional Quackery*

THE popular appeal, through the press and commercial advertisements, of matters relating to food and health has elicited wide popularization, and dissemination of information developed by scientific investigations in the field of nutrition. As a result, we have become as a nation "food conscious." The great popular demand for information about foods and dieting, together with the erroneous idea in many cases that nearly every disease and ailment can be cured by some system or other of dieting, has offered an unusually attractive opportunity for exploitation. During the last few years there has been an increasing amount of publicity, and exploitation of false and harmful statements foisted upon the public by dietary quacks, faddists, and so-called nutrition "experts." Some of them may be honest, but ignorant and misguided enthusiasts and fanatics. Many of them, however, are fakers, so-called "doctors," with little or no recognized professional standing, motivated by personal gain, who have selected this fruitful field for preying on the public.

Notwithstanding the large amount of popular scientific knowledge available in nutrition, the successful preying on the public by faddists and quacks has assumed alarming proportions. Influenced by promises, fanciful appeals to fear, and suggestion, many choose the courses offered by the faddist in preference to the advice of reputable authorities. Unfortunately, a large proportion of the population is unable to discriminate between the claims of the charlatan or ignorant faddist and those of investigators whose scientific contributions and knowledge have gained for them the position of outstanding authorities. Even physicians, nurses, teachers, medical students, and dietitians are numbered among the victims. Aside from mulcting thousands who can ill afford it, the exploitation of these dietary notions is a menace to public health, causing serious injury as a consequence of relying upon these false dietary systems for alleviating their ailments instead of seeking the timely aid of reliable medical assistance.

The most common subjects of the appeal which these quacks make are food combinations and incompatibilities. Based on entirely false representations, vicious systems of dieting which do much injury are given in books and pamphlets sold under high pressure salesmanship.

One of the most common and extensively proclaimed nutritional fallacies is that proteins and starches are incompatible and should be separated into distinct and separate meals. Based largely on this idea a system of dieting has been developed and featured in books and syndicated newspaper columns. A monthly magazine is devoted to this system. It is maintained that because starches require an alkaline medium for their digestion and proteins an acid medium, an antagonistic effect is developed when both are taken

* Report of the Committee on Nutritional Problems.

together which seriously interferes with the digestion of each of these classes of foods. One can look in vain through the writings of authorities in textbooks and journals for any scientific experimental data in support of this idea. The proponents ignore the fact that a large proportion of our staple articles of food contain both starch and protein. Adherence to this system would necessarily eliminate from the diet practically all products made from cereals and grains, such as bread, crackers, macaroni, cakes, pastries, etc. It would also exclude potatoes, beans, peas, and many other important foods. The cereal grains, wheat, rye, barley, oats, and corn, range in their protein content from 9 to 12 per cent or higher, and contain also about 60 per cent starch. Rice contains about 7 per cent protein and upward to 80 per cent of starch. Beans and peas are rich sources of both protein and starch, containing from 20 to 25 per cent protein and 40 to 50 per cent starch. Chestnuts range from 9 to 10 per cent protein and 20 to 30 per cent starch.

This absurd system would ban the time honored combinations which for generations have been recognized as wholesome and healthful, such as meat and potatoes, bread and milk, bread and cheese.

Investigators and authorities in the field of nutrition are practically unanimous in the opinion that there is no incompatibility between starches and proteins in the diet.

> Faddists have hit upon the idea that we should not eat starches and proteins at the same meal because starch digestion is interrupted in the acid stomach, acid being essential to peptic or stomach digestion of proteins. This is a fallacy. There is no significance in this interruption. The cat, dog, and cow have no starch-digesting ferment in their saliva, but they thrive on foods rich in starches. All their starch digestion takes place after the food has left the stomach and reaches the small intestine. Nature did not put these animals at a disadvantage in this respect. *McCollum and Becker.*[1]
>
> Much of what is said by food faddists relative to combinations of protein and carbohydrate is fallacy without any basis in scientific study. *Fishbein.*[2]

It has been shown that a mixture of carbohydrate and protein foods is discharged from the stomach in shorter time than protein alone, indicating that

> . . . the addition of carbohydrate to the diet accelerates digestion and the discharge of the gastric contents. *Cannon.*[3]

Studies by Rehfuss, Hawk, and others [4] on human gastric digestion in the stomach involving more than 1,000 studies on 200 normal men, in which a great variety of protein and starch combinations was used, failed to produce any evidence whatever of the incompatibility of these 2 classes of foods. In order to meet criticisms of the food faddists, that different results would have been obtained had the studies been made with chronic invalids instead of normal individuals, Rehfuss [5] has more recently extended the investigations to include a cross-section of medical invalids representing almost every variety of chronic illness encountered in a medical work service. Meat was used to represent protein, and potatoes to represent starch. The results of these studies involving hundreds of observations and representing a year's work in medical service, again demonstrated:

> . . . the absolute inaccuracy of the statement that proteins and carbohydrates are incompatible in the stomach. . . . There is no evidence either in the literature or in my investigation to lead me to believe that proteins and carbohydrates are incompatible in the stomach. The danger of such teaching based on a lack of scientific evidence is manifest, and while it may be true that many individuals overeat and are presumably better by a reduction of carbohydrates, the unqualified acceptance of such a teaching can lead to the occurrence of serious malnutrition as well as to a lighting of tuberculosis and old infections.

We are also told to eat but one kind of starch at a time and one kind of protein. This is obviously so devoid of any scientific reason that it merits but a mere passing reference. All starches yield on digestion the same simple sugar, glucose. The end product is the same irrespective of the source of the starch. As to proteins, to eat one kind at a time is just what one should not do. Proteins differ in their nutritive value depending on their amino acid composition. Some proteins are lacking or deficient in certain amino acids which are nutritionally indispensable.

Nutritionally deficient or incomplete proteins should be supplemented with other proteins so that the amino acids lacking in the one will be supplied by the others in order to have an assortment of amino acids adequate to meet the requirements of the body. For example, the endosperm proteins of the cereal grains in general are deficient in lysine and tryptophane, two amino acids indispensable for growth and normal nutrition. The proteins of meat, milk, eggs, and nuts, on the other hand, are excellent sources of these amino acids. The value of the cereal proteins in the diet is enhanced by inclusion of the latter protein foods in the diet. Instead of an incompatibility we have a supplementation.

Acidosis is a term that is frequently and effectively used by the purveyors of food fallacies in the exploitation of their books, literature, and dietetic systems. Nearly all diseases that afflict mankind can be found enumerated as the result of acidosis caused by eating "acid foods." Elaborate menus are offered for "alkali-forming" meals, and systems of dieting which can be had by purchasing their books or enlisting their services and special courses. The claim that acidosis, with all its dire effects, will result from eating bread and meat or certain combination of foods, such as proteins and starches, or fruits and starches, is entirely unsupported by scientific evidence.

Acidosis is usually a condition attending certain diseases, such as diabetes or kidney diseases, involving a faulty metabolism of the body.

There is no evidence that a preponderantly acid diet is injurious.

That the body reaction remains practically unaltered even when a wide range of amount of acid or base is ingested has been pointed out by Henderson.[6]

McCollum and Hoagland[7] found that long continued excess of acid-forming elements in the diet did not lead to any apparent injury.

The Committee on Foods of the American Medical Association in one of its recent decisions states that

> . . . acidosis is a name for a morbid condition of diminution in the reserve supply of fixed alkali in the blood and body fluids. Most people have no conception of the true meaning of the word and are quite likely to confuse it with gastric hyperacidity or "acid stomach," or to conceive of it as "acid blood," a condition which would be incompatible with life. The term "acidosis" is so little understood that its use in any advertising except that restricted to the medical profession is misleading and consequently disapproved.

As stated by Stone,[8] the term acidosis has become a popular fancy.

> . . . The belief that acid-ash foods are responsible for a variety of common symptoms such as gastric hyperacidity, lassitude, biliousness, acid mouths, headaches, nephritis, and high blood pressure carries one far afield. . . . The importance of the acid-ash diet as a factor in the etiology of disease has been overestimated.

Mariott[9] believes that ". . . acidosis is a rare condition and there is no good evidence that the regular taking of large amounts of alkaline-ash foods is of any special benefit." In his experience there is no evidence ". . . that an alkaline-ash dietary

can effect the reduction of established arterial hypertension."

Arthritis is a disease which is commonly emphasized as resulting from improper food combinations and which readily yields to the particular dietetic system which is exploited. We are told to omit "acid fruits and vegetables"; to partake of only one type of food substance at a meal; to alter the acid-base balance of the diet; to use a low protein and carbohydrate diet. Carbohydrate restriction is based on a dietary rationale, the correctness of which has not been satisfactorily demonstrated by scientifically controlled experiments. The commonly so-called acid fruits, such as oranges, tomatoes, and grapefruit, contain weak organic acids which are easily oxidized in the body. These acids are chiefly present as salts of inorganic bases which are left in the blood as alkaline carbonates and really serve as available alkali to the body. The fallacy and inadequacy of these claims have been recently pointed out by Bauer.[10]

> One need make no conscious effort to maintain a basic diet in an arthritic person any more than in a normal one. . . . The prescribing of a low protein diet is a relic from the days when rheumatoid arthritis was confused with gout. There is no justification for the limitation of proteins in the dietary of a patient with rheumatoid arthritis. There are many reasons why it should be liberal.

Another fallacy which finds a prominent place in the armamentarium of the nutritional quack is that acid fruits should not be eaten at the same time with carbohydrate foods, including breads and cereal products, potatoes, squash, and sugars of every kind. These foods, we are reminded, require an alkaline medium for their digestion, and without the alkaline reaction of the saliva they would never be digested at all. This view ignores the well known fact that only a part of the carbohydrate digestion occurs in the mouth and stomach as a result of the action of ptyalin in the saliva, and that there are active amylolytic enzymes in the intestines where carbohydrate digestion proceeds to completion. In fact, starch digestion can proceed favorably in a medium that is practically neutral. The relatively small amounts of the weak organic acids in fruits can have little or no significance in any interruption of starch digestion in the stomach where the hydrochloric acid of the gastric juice is many times stronger than the fruit acids.

Emphasis is laid by "experts" and exploiters of preparations offered for sale on the claim that the use of common table salt is responsible for Bright's disease, cancer, tuberculosis, high blood pressure, and other diseases and ailments.

The surmise that salt has some relation to high blood pressure has influenced to some extent the treatment of this disease. However, the work of O'Hare and Walker[11] lends no support to such a view. No relation was found to hold between blood pressure and the chlorides of the blood and plasma, and no effect on the systolic and diastolic levels was observed during wide variations in the amounts (0.5 gm. to 4 gm. daily) of salt taken in high pressure cases without nephritis. McLester,[12] in a series of controlled observations with patients having hypertension, was unable to note any improvement when a so-called "salt-free" diet was used. He believes, however, that in cases of hypertension complicated with nephritis the salt intake should be restricted "to that contained in the food as it reaches the table." While the avoidance of large quantities of salt in the diet of patients suffering from certain diseases may be advisable, there is no evidence that salt in the quantities generally used in the diet results in the development of such

diseases as mentioned or is attended by any harmful effects.

We are told to avoid the use of dark meats or to limit our meat consumption to fish and fowl. The statement that white meat is less harmful than dark meat has no basis in fact.[12] This belief goes back to the days when gout was confused with other forms of arthritis, and that foods rich in purines yield uric acid with resulting gout, rheumatism, and acidosis. With the exception of the glandular organs, liver, sweetbreads, and kidneys, dark meat contains no more purine bases than does white meat. Fish and fowl contain as much, or more, uric acid-forming bases as beef, veal, lamb, and pork.[12]

It has been proved by numerous investigators [13, 14, 15, 16, 17, 18, 19] that the white of eggs is much less digestible when raw than when cooked. There is even evidence [20, 21, 22, 23] that raw egg white when fed to experimental animals will invariably produce toxic symptoms. Nevertheless, it is still not uncommon to find physicians and nurses prescribing raw eggs for invalids in the belief that they are more digestible and more easily assimilated than when cooked.

Among the dicta of faddists and food charlatans are many so fanciful and absurd that to the person of ordinary intelligence they need no refutation.

As illustrations of such fallacies may be mentioned the following: Tomatoes cause cancer; meat makes one sensuous and belligerent; mixtures of incompatible foods explode in the stomach; fish is a good brain food; garlic "purifies" the blood; cream should not be eaten with lobster, strawberries or pickles; fish and celery should not be eaten together; "dead foods" should be avoided in the diet.

Many different systems of dieting and treatments are advocated for weight reduction, some of which are merely scientifically unsound; others are positively harmful and dangerous. Many, eager to conform to the current prevailing fashion of being slender, adopt short-cuts to reduction advertised by food promoters and those interested only in their own personal gains, have done themselves harm, bringing on weakness, nervous irritability, and anemia. Particularly to be deprecated is the use of salts and dangerous drugs. In reduction treatment the first consideration is curtailment of calorie intake, with the object of accomplishing a gradual loss of weight without sacrificing the amount of protein, vitamins, and mineral salts required for normal body needs, nor even of the calories needed as a source of energy for body temperature and for production of work. Many reducing menus are erroneously based on elimination from the diet of individual foods, such as potatoes, rice, cereal products, and butter. In order to assure an adequate balance of food factors essential for health and normal nutrition, it should be remembered that it is not the kind of food that should be restricted, but rather the quantity. As Mendel [24] has aptly stated, "Sanity in diet calls for moderation in eating rather than exclusion of any wholesome food. Even the slenderest persons need some energy for their daily undertakings."

It is only by a process of education that the public can be enabled to discriminate between the fallacies proclaimed by the food faddists and quacks, and the sound principles of nutrition established by reliable investigators. Public health officials are in an excellent position to render a great service in educating the general public along these lines, and in waging a concerted attack on the operations of these enemies of public health.

There is plenty of reliable literature available which can be effectively used in conducting such a campaign of education.

In addition to the references already cited in this report, there are given others [25, 26, 27, 28, 29, 30] which excellently cover the subject.

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Standard Methods of Vitamin D Bio-Assay of Milk

IT is obvious that the appointment by the Association of Official Agricultural Chemists of an associate referee, Dr. Walter C. Russell, to study the problem of a standard method for bio-assay of vitamin D in milk constitutes a duplication of effort in our two societies. Accordingly, your committee proposes to collaborate in so far as possible with the associate referee of the other association and to draw its own conclusions from the evidence which has been collected by one of us for joint information. The data already collected by Dr. Russell which will soon be considered by your committee independently pertain to the 5 following topics:

1. Duration of the test period
2. Method of feeding the milk, comparing the results obtained by incorporating the milk in the basal diet, with those obtained by feeding it separately from the basal diet
3. Suitability of basal diets for control animals which receive cod liver oil
4. Methods of preserving milk during the period of test
5. The suitability of a reference oil as a standard for comparison

It is planned to continue further collaborative work on all 5 of these items, and upon such others as appear to your committee to be pertinent. The bio-assay of vitamin D milk should obviously follow the principles of the method laid down in the United States Pharmacopeia, which became official on January 1, 1935.

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Pneumoconiosis

THE current use of a variety of classifications for the roentgenological appearances of silicotic lesions is making it difficult to correlate the results of a rapidly increasing number of observers. In South Africa, where silicosis is a product of a single industry, and where the problem is handled almost exclusively by a bureau of the government, the classification that has evolved is entirely adequate for their purposes; but in a highly industrialized country silicosis develops in a great variety of occupations. A diagnosis may be required of a physician in a plant, in a public health office, in a sanatorium, or in the physician's private office. Sometimes he may have had little experience in the interpretation of roentgenograms of the chest, and will be forced to lean heavily on the interpretation of a roentgenologist.

The legal aspects of the problem have placed grave responsibilities on the medical profession and have often been a source of embarrassment. Only the physician who has examined the subject has obtained an occupational history of an adequate exposure to silica dust, and has before him a suitable roentgenogram of the chest, should make the diagnosis of silicosis. The roentgenologist, not in possession of these facts, can merely state whether the shadows which he sees in a film are consistent with this diagnosis.

If objective terms descriptive of the type of pathological changes could be generally adopted, material progress would result. The clinician would not have to accept a diagnosis from the roentgenologist; general students of the disease would be able to correlate the findings of the various observers; and more accurate definition of roentgenograms would be available for medicolegal purposes. Roentgenograms of the chest, which are notoriously difficult to reproduce as illustrations, could be described in word pictures capable of interpretation in the light of the personal knowledge of the observer. An error in diagnosis need not necessarily be passed on to others not in possession of the original film.

With these obstacles in mind, a committee has been at work for the past 2 years preparing a tabulation of the various lesions of silicosis together with terms that attempt to depict the character of the shadows cast on an X-ray film by these lesions. This report is submitted to invite criticism, in the hope that the terms suggested, or others of a similar nature, will be generally adopted.

It should be distinctly understood that the tabulation which follows applies only to silicosis, that form of pneumoconiosis which results from the inhalation of dust with a high silica (SiO_2) content.* Other forms, like asbestosis, are excluded from this consideration because their pathology is essentially different from that of silicosis.

The tabulation contains two columns: On the left are the roentgenological appearances and on the right are the corresponding pathological lesions. There is further subdivision to

* Some of the non-siliceous components of certain industrial dusts seem to modify the pathological reactions, but the character of shadows cast by these modified lesions is not sufficiently defined at the present time to include them in the tabulation. Later, when more information has accumulated, certain other terms may have to be included.

HEALTHY LUNGS AND ADNEXA

Roentgenological appearances	*Histological appearances*
1. *Healthy lungs*—As defined by the N.T.A. Committee report.	1. Essentially the normal tissues of the vascular tree, the mediastinum, the bronchi, and trachea.
2. Irregular exaggeration of the linear markings, with possibly some beading confined to the trunks.	2. Cellular connective tissue proliferation about lymphatic trunks in the walls of vessels and bronchi. Beading may be due to various causes, as blood vessels seen end on, arteriosclerosis, minute areas of fibrosis in lymphoid tissues along the trunks.
3. Increased root shadow.	3. Cellular reaction in the tracheo-bronchial lymph nodes with extensions along afferent lymphatic trunks.

These changes come within normal variations when not accompanied by recognized organic disease.

SIMPLE SILICOSIS

4. *Nodulation*—Discrete shadows not exceeding 6 mm. in diameter, tending to uniformity in size, density, and bilateral distribution, with well defined borders surrounded by apparently normal lung shadows. The outer and lower lung fields characteristically show fewer nodules.	4. Circumscribed nodules of hyaline fibrosis located in the parenchyma of the lung. Occasionally some of these nodules may show microscopic foci of central necrosis.
5. Conglomerate shadows that appear to result from a combination or consolidation of nodulation usually with associated emphysema manifested by— a. Localized increased transparency of the lung with loss of fine detail b. Intensification of the trunk shadows by contrast. c. Depression of the domes with possible tendency toward individualization of the costal components of the diaphragm. d. Lateral view: Increase in the preaortic and retrocardiac space with exaggerated backward bowing of the spine. Widening of the spaces between the ribs may or may not be present.	5. The result of coalescence of discrete nodules; an area in which the nodules are closely packed and most of the intervening lung is replaced by more or less hyaline fibrous tissue. The lung architecture is partially obscured. No demonstrable evidence of infection. Emphysema is a compensatory dilatation of the air spaces with or without thickening of the septa.

describe the appearances of: (1) healthy lungs, (2) the uncomplicated silicotic lung, and (3) lung of silicosis with infection. The changes described under the first division are those compatible with a state of good health; and while they *may* be produced by the inhalation of relatively small amounts of silica dust, they are not sufficiently characteristic or advanced to substantiate a diagnosis of silicosis. Similar or identical appearances may also result from the inhalation of non-siliceous dusts, from certain infections, from cardio-vascular disease, and from certain other rare conditions. The changes involved are for the most part confined to the lymphatics and peri-lymphatic connective tissues and do not often affect the parenchyma of the lung. Since, by definition, silicosis is a disease characterized by nodular fibrosis in the parenchyma of the lung, these alterations, even when they may have been caused by inhaled silica, do not constitute a basis for a diagnosis

SILICOSIS WITH INFECTION

The characteristic appearances described under simple silicosis are modified by infection as follows:

6. Localized discrete densities and/or string-like shadows accompanying those of simple silicosis described above.	6. Strands of fibrous tissue, often along trunks and septa, with or without areas of calcification; indicative of "healed" infection.
7. *Mottling*—Shadows varying in size with ill-defined borders and lacking uniformity in density and distribution, accompanying simple silicosis.	7. a. Areas of broncho-pneumonia with or without caseation, *i.e.*, acute infection. b. Lobular areas of proliferative reaction with or without caseation, *i.e.*, chronic infection.
8. *Soft nodulation*—The nodular shadows described under simple silicosis, 4, have now assumed fuzzy borders and/or irregularities in distribution. The change may or may not accompany the simple mottling of 7.	8. Perinodular cellular reaction either exudative or proliferative in character.
9. *Massive shadows* of homogeneous density not of pleural origin symmetrically or asymmetrically distributed.	9. Extensive areas of fibrosis probably due to organized pneumonia of tuberculous or non-tuberculous origin superimposed upon a coexisting silicotic process. Outlines of normal structures may be partially destroyed.

of silicosis. The second group covers the discrete and conglomerate nodular fibrotic reactions of simple silicosis. The last group deals with silicosis complicated by infection. In the majority of instances the infecting organism is the tubercle bacillus, but the classification is sufficiently broad to include other types of infection. Certain criteria by which one attempts to differentiate various forms of infection will be discussed.

COMMENT

For the first group of appearances we have adopted the nomenclature of the National Tuberculosis Association Committee and described the lungs as *healthy* rather than as *normal*, as a perfectly normal adult human being is a great rarity. For a description of the roentgenological appearance of the healthy chest the reader should consult a paper by Pancoast, Baetjer, and Dunham in the *American Review of Tuberculosis* for 1927 (vol. 15, pp. 429–471).

As already mentioned under 2, *Irregular exaggeration of the linear markings with possibly some beading*, belongs in the healthy chest group even when found in persons with a history of considerable exposure to silica, for such changes are nonspecific in character and they do not involve the parenchyma of the lung. Silicosis as a clinical disease begins only when the lung proper is affected. Likewise, under 3, *Increased root shadow* may be of nonspecific origin and hence is not diagnostic. In the *early* stages of silicosis the mediastinal shadow may be widened, owing to the enlargement of the tracheo-bronchial lymph nodes from accumulated dust and cellular reaction to it; later, when specific fibrosis develops, the tissues generally contract and the nodes decrease in size. The changes described under 2 and 3 may be caused by many forms of irritation; if they are due to silica they are identifiable only by miscroscopic examination. They do not apparently interfere with respiratory function, and they are not of diagnostic significance.

The second group of changes is

limited to *simple silicosis* uncomplicated by demonstrable signs of infection. This condition is characterized by the presence of small, discrete nodules of fibrous tissue disseminated throughout the functional parts of both lungs. The lesions and the shadows cast by them tend to be spherical, hard, sharply defined, and vary in size from 2 to 6 mm. While the distribution is usually uniform throughout both lungs, the extreme apices and the outer portion of the bases are frequently uninvolved. In less advanced cases the nodules remain discrete and separated by air-containing tissue. Irvine has aptly compared the shadows observed in the roentgenogram of the silicotic lung to those cast by a tree. In the earliest stages, when the reaction to silica is confined to the perilymphatic connective tissues, producing an accentuation of the linear markings, the shadow cast is that of a leafless tree. As small nodules begin to appear in the parenchyma of the lung, the tree begins to bud and the shadow of its branches is less clearly defined. When the tree is in full leaf, the stage of advanced nodulation, the shadow of the branches is completely obscured. Previous classifications have been concerned largely with the degree of nodulation as determined by the size and number of the nodules in the lung. We will not attempt to deal with this problem here, but choose to leave the "stage" of the silicosis to the internist, who has physical findings as well as a roentgenogram at his command.

Number 5 deals with the *conglomerate shadows* of simple silicosis, which appear to develop from a combination or consolidation of discrete nodules. The resultant lesion and the shadow that it casts are often difficult to distinguish from the *massive shadows* of silicosis with infection, 9. It is generally assumed that conglomeration results from accidental overlapping and fusion of discrete nodules when they become very numerous; but since conglomeration is usually a localized affair and does not occur in the same position of the lung of every individual, it is logical to inquire why the nodules happen to fuse in one portion of the lung and not in others. Microscopic examination of the tissues from such areas reveals no evidence of active infection. The nodules seem to be much closer together than in other portions of the lung, they are less uniform in size; and they are usually embedded in a matrix of diffuse fibrous tissue having the same characteristic hyaline appearance as that forming the nodules themselves. It seems probable that conglomeration may have occurred because the portion of the lung in question was previously damaged by a localized inflammatory process occurring before or during the early period of dust exposure. Because the tissue was injured, more dust would tend to accumulate in the area, the nodules would develop irregularly and would frequently be very close together. The silica lodging in preëxisting granulation or scar tissue would exert its characteristic effect, and a diffuse hyalinization would result. This explanation for conglomerate reaction is at present hypothetical; proof will come from long continued serial roentgenographic studies of groups of persons exposed to silica dust and from the chance autopsy that may be obtainable. To differentiate *conglomerate shadows* from the *massive shadows* of infection, 9, reliance must be placed upon the absence of change in size and character of the shadows in serial films taken over an extended period, and upon the clinical findings in the case.

Emphysema is usually associated with far advanced silicosis and it is particularly liable to complicate conglomerate nodulation. It occurs in the

immediate vicinity of the conglomeration as a result of the distortion produced by contracting scar tissue; there is also a generalized "compensatory" emphysema found along the borders of the lung, particularly at the bases. The latter type is also common in far advanced generalized nodulation.

In the last group, *silicosis with infection,* are included all cases with detectable evidence of infection whether active or inactive. In this respect we depart from the South African procedure, which includes here only active infection. The difficulty of determining activity, particularly in the silicotic subject, is our chief reason for this arrangement.

Number 6 covers foci of healed infection. Identification of such changes depends upon the same criteria that are generally employed in otherwise normal individuals. In the silicotic subject the shadows usually occur upon a background of generalized nodulation, although in some cases there may be a distinct tendency toward excessive nodulation in the immediate vicinity of the scars left by the infection. Where the exposure to dust has been limited, the major evidence of nodulation may occur about the foci of healed infection with much less reaction in the remainder of the lung. The string-like shadows of healed fibroid tuberculosis are not difficult to interpret if they occur in the classical location, *i.e.*, in the upper third of the lung. In the lower lung they present a problem whose solution depends largely upon the experience of the roentgenologist.

The term *mottling,* 7, we have reserved to describe the shadows of infectious lesions in contradistinction to *nodulation,* which is restricted to those of the silicotic dust nodule. It is essential that this distinction be appreciated and recorded in the terminology. In tuberculosis, mottling is due to bronchogenic or aspiration foci of disease which exhibit a characteristic clustered arrangement. The lesions may be exudative (acute) or productive (chronic) in type; the difference will be registered on the roentgenogram by a mottling which is fluffy and ill-defined, or hard and sharply defined as the case may be. The distribution of the mottled foci, together with the presence of large foci of older disease interpreted as tuberculosis, and clinical and laboratory findings establish the character of the infection. Mottling due to chronic infection that has developed previous to or simultaneously with the relatively early periods of dust exposure may exhibit little or no effect from the inhaled silica for many years. In non-tuberculous broncho-pneumonias the large chronic foci are absent, and the disseminate mottling may involve different parts of one lung or of both lungs. In many instances the nature of the infection must be established by serial examination over considerable periods of time and by careful correlation with clinical and bacteriological findings.

Soft nodulation, 8, is a term that has been coined to describe a rather uncommon combination of silicosis with infection, usually tuberculous. The ordinary hard, sharply defined nodular shadows of simple silicosis, under these circumstances, appear to have enlarged and lost definition. Their borders are now fuzzy and blend imperceptibly with the surrounding lung structure. Such lesions generally occur in association with localized conglomerate shadows in the apex or other portions of the lung. Histologically the infection appears to have localized in and about preëxisting silicotic nodules so that each is surrounded by a zone of exudative or productive cellular reaction.

Massive shadows of homogenous density, 9, are cast by the areas of combined silicosis and infection, usually

chronic in nature. The two processes appear to have developed simultaneously, and unusual amounts of dust accumulate in the diseased areas. Generalized nodulation usually occurs throughout the remainder of the lungs. Pleural densities can be differentiated in stereoroentgenograms, and by over-exposure it often becomes possible to penetrate the extremely dense intrapulmonary areas and analyze their internal structure. Not infrequently cavities may be visualized that were completely overlooked with the usual technic. When due to tuberculosis, such lesions are often bilaterally symmetrical. If the process extends to the pleural surface, a tuberculous etiology is postulated, while other infections are more often deep-seated.

Histological examination of such lesions shows conglomerations of simple nodules embedded in masses of more or less perfectly organized granulation tissue. Often the fibrous tissue has undergone the same peculiar hyalinization that characterizes the interior of the silicotic nodule. Usually the outlines of the original lung architecture are completely destroyed. Manifestations of infection depend upon the nature of the process—if tuberculous, there will be foci of caseation and possibly small cavities. Calcification is not infrequent. If the process is inactive, the presence of fibrous tubercles which do not exhibit the hyalinization of silicosis may be present. The occurrence of giant cells is helpful. A partially organized nontuberculous pneumonia usually contains foci of acute exudation of variable size. Clinically such unresolved pneumonias frequently exhibit periods of exacerbation followed by regression. They may be due to a great variety of organisms, including the Friedlander group and oral anaerobes. Where all manifestations of activity have disappeared, the lesion is probably best classified as a conglomerate shadow of simple silicosis, 5.

The differentiation between these *massive shadows* of infectious origin and the conglomerate shadows of far advanced silicosis is difficult and not always possible. Repeated reëxamination of the patient for evidence of change in the roentgenographic appearance of the lesion, penetration of the massive areas by over-exposure to analyze its internal structure, the clinical behavior of the patient, and repeated bacteriological examination of the sputum may all be necessary to determine whether an active infection is present.

It is hoped that the suggested terminology will receive a practical test. If others find it usable, the advantage of standard descriptive terms should outweigh the conservatism of those who are already using classifications of their own.

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H. K. PANCOAST
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Skin Irritants*

THIS report is in the form of an annotated bibliography of some 782 papers or publications dealing with industrial skin irritants. This is a continuation of similar former reports and covers the period of 1929–1934 inclusive. There is a somewhat detailed discussion of the subject as developed in these papers, with an evaluation of the work as reported, and with an indication of the most interesting and important progress and of new developments in the field.

The bibliography is classified under the following heads:

1. General subjects, including statistical data, general prophylaxis, diagnostic tests and treatment, with special consideration of eczema, and of allergy or sensitization
2. Trade dermatoses
3. Physical irritants
4. Animal and animal product irritants
5. Plant and plant product irritants
6. Microörganisms
7. Inorganic chemical irritants
8. Organic chemical irritants
9. Industrial skin cancers and carcinogenic factors, with a number of papers presenting experimental studies on these lines
10. Medico-legal considerations

The bibliography is supplied with a completely cross-referenced author and subject index.

NOTE: The abstracts on which the report is based are filed (645 of them) in the office of the Committee Chairman in the Hygiene Department of the University of Pennsylvania and are available to interested members.

HENRY FIELD SMYTH, *Chairman*

* Abstract of the Report of the Committee. The complete Report will be published by the U. S. Public Health Service.

Standard Practices for Compensation in Occupational Diseases

IN 1929 a preliminary report was submitted to the Industrial Hygiene Section, at Minneapolis, and was distributed in mimeographed form to members of the Section. This material was again revised and submitted to the Committee on Research and Standards, which approved the project for publication. By a grant from the Chemical Foundation, this was done in 1931 and made available to the general public.

This report consisted of a compilation of occupational disease legislation, covering Europe and the Americas. From year to year the committee has reported on the changes in occupational disease legislation, so that the original report of 1931 has been kept up-to-date by these supplementary reports.

In addition to this primary study, the committee collaborated with the Committee on Lead Poisoning in 1932, and the Committee on Pneumoconiosis in 1931, in the preparation of standard practices in the compensation for these two diseases.

The committee also prepared an extensive report on the definition of occupational disease in 1932 for the National Committee on Nomenclature.

While the primary objectives of this committee were to study and establish standard practices, it feels that it has achieved only the first of its two purposes. It was hoped that the compilation of occupational disease legislation would be utilized by legislative and social agencies, employees' insurance companies, as well as labor organizations who were interested in improving or changing existing legislation to conform with modern progress and standards. We have been disappointed. It would seem that all the data accumulated by our committee on existing laws, studies, surveys, bills, etc., over the past few years, have not been utilized by law making bodies. This may be seen in the perfect flood of bills that have been introduced in a number of states, all of which, with the exception of those in North Carolina, have disregarded established principles of occupational disease compensation. New York, for example, has passed a blanket occupational disease law which disregards the experience of many countries, like New Zealand, which has changed from a Blanket Law to a Schedule Law.

The failure of this committee to achieve its objective is not due to the nature of the material and reports, but to its make-up. This committee cannot hope to gain a hearing from legislative bodies unless it has the authority that comes from an enlarged representation. Without this it is futile for the committee to continue and it would be well for it to be dismissed. However, if this section feels that it is desirable to pursue this objective of establishing standard practices, it will be necessary to enlarge its representation to include the various interests involved—the employer, the worker, the government, the university, and other social agencies.

We request therefore that the committee be directed to make a preliminary report at the next meeting with a view to determining the feasibility of creating as a projecting aim of this committee and section a central agency representative of all groups in-

terested in the improvement of standard practices in the compensation for occupational disease. This central agency would concern itself with the promotion of education, legislation, research, and the creation of a panel of qualified medical and non-medical experts in industrial hygiene for medico-legal purposes.

HENRY H. KESSLER, *Chairman*
BERNARD S. COLEMAN
EMERY R. HAYHURST
GEORGE M. PRICE
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Ventilation and Atmospheric Pollution

Part I

Ventilation and Atmospheric Pollution

THE following was approved by the committee and adopted by the Section on Industrial Hygiene at the Milwaukee Meeting as an outline of working standards for atmospheric and space environments for the maintenance of comfort, health, and efficiency, assuming altitudes not exceeding 10,000 feet above sea level *:

1. Cool rather than hot †
2. Dry rather than damp †
3. Still ‡ or moving depending upon physical activity †
4. Some diversity in temperature—time and space—rather than uniformity and monotony †
5. Foot level as warm as head level
6. Radiant source as an item in heating, preferred
7. Graded differences between air-conditioned quarters and outer air, depending upon the length of stay in-doors, *i.e.*, less difference for brief stays
8. Odor-comfort
9. Minimum area for adult person of 25 sq. ft. and cubitage of 200 cu. ft.
10. Reduction of obnoxious dusts, bacteria, fumes, vapors and gases to their sub-danger thresholds
11. Satisfactory primary sense impression upon entering the room or space
12. Maintenance of comfortable conditions during occupancy (room comfort impression)
13. Sufficient replacement of "foul air" with "fresh air" to meet odor-comfort requirements
14. Ionization and actinic ray effects on air itself to be disregarded for the present
15. Intelligent supervision

The items of compressed air, ultra-violet rays, X-rays, and radium exposures are not considered here.

EMERY R. HAYHURST, *Chairman*
PHILIP DRINKER
LEONARD GREENBURG
WILLIAM J. MCCONNELL
CAREY P. MCCORD

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* Agreements as to the majority of the exact specifications, of interest to engineers, were summarized in the previous report of this Committee (*Year Book*, A.P.H.A., 1934–1935, pp. 108–112).

† Adapted from the British Health of Munition Workers' Committee, *Memorandum No. 9*, 1916.

‡ "Still" implies air motion under 25 ft. per minute.

Part II

Standard Methods for the Examination of the Air

THE last report to the Association on Standard Methods for the Examination of the Air, known as the "Fourth Supplementary," was made at the New Orleans Meeting, October 27, 1919, by a committee consisting of C.-E. A. Winslow, *Chairman*, T. R. Crowder, W. P. Mason, E. B. Phelps, and C. G. Whipple.

At the Pasadena Meeting, in 1934, the Association Committee on Research and Standards referred to the Section on Industrial Hygiene the matter of bringing this subject up to date. Further instructions were that the Laboratory Section and the Public Health Engineering Section also name representatives for the purpose. Accordingly, the Section on Industrial Hygiene referred the matter to its Standing Committee on Ventilation and Atmospheric Pollution, the personnel of which is named in Part I. Through the good offices of the secretaries of the other named Sections, the two following gentlemen accepted invitations which completed the present committee: G. L. A. Ruehle, of the Food and Drug Administration, U. S. Department of Agriculture, to represent the Laboratory Section; and Harry B. Meller, Head, Air Pollution Investigation, Mellon Institute for Industrial Research, to represent the Public Health Engineering Section. For convenience, this newly constituted committee was named the "Special Committee on Standard Methods for the Examination of the Air."

The plan of procedure adopted was to divide the subject into 4 parts: Physical, Chemical, Dust, and Bacteriological. Specialists in these various fields were then sought and requested to draft the respective reports wanted.

The committee is especially indebted to the following gentlemen for reports on 3 of the 4 divisions (as submitted below):

C. P. Yaglou, Department of Industrial Hygiene, Harvard School of Public Health, Boston, Mass.

Warren A. Cook, Chief Industrial Hygienist, Connecticut State Department of Health, Hartford, Conn.

J. J. Bloomfield, Office of Industrial Hygiene and Sanitation, U. S. Public Health Service, Washington, D. C.

Their reports, it is believed, take up, by inclusion and references, the most immediately needed features and with a minimum of overlapping.

The fourth division—Bacteriological—has still to be drafted, and the committee hopes to be able to secure the services of a well known investigator to assume the task and present his report in time for the next Annual Meeting of the Association, to be held in New Orleans, 1936.

At the Milwaukee meeting (October, 1935), it was further decided to include the original drafters in the personnel of the committee and ask each to head-up a sub-committee to keep in touch with the developments in his special field and to report at intervals, as seems desirable. Comments and suggestions are invited, and may be taken up with any member of the committee as befits the subject matter.

EMERY R. HAYHURST, *Chairman*
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Physical Procedures in Air Analysis

Instruments and Methods for Recording Thermal Factors Affecting Human Comfort

C. P. YAGLOU

1. Temperature: Mercury or alcohol thermometers capable of recording temperature within 0.5° F.
2. Humidity: Sling psychrometer
3. Air movement: Kata thermometer [1] or heated thermometer [2]
4. Radiation: Thermo-integrator [3, 4] or heated globe thermometer [5]
5. Combined effects of 1, 3, and 4, under conditions of minimal perspiration: thermo-integrator or heated dry globe thermometer
6. Combined effects of 1, 2, and 3 under conditions of maximal perspiration: effective temperature index [6] or heated wet-globe thermometer

Comfort in so far as heat or cold is concerned is associated with a normal heat balance between heat production and heat loss, at normal body temperature, and without sweating. Four of the basic factors affecting human comfort are air temperature, radiation, air movement, and humidity. No one of these is independent of the others, but their effects are quite different under conditions of minimal or maximal perspiration.

In men at rest under comfortable air conditions, the total metabolism remains constant, but the proportion of heat loss by radiation and convection may vary depending upon the external conditions. In still air, radiation accounts for about 45 per cent of the total heat loss, convection for 30 per cent, and evaporation for 25 per cent. About 11 per cent of the total heat loss takes place by evaporation from the lungs and 14 per cent from the skin. The former depends on the relative humidity, varying from about 12 per cent at 20 per cent R.H. to 9 per cent at 100 per cent R.H. Evaporation through the skin is limited to the output of water. Insensible perspiration is now believed to be chiefly a filtration of water from the capillaries, so that alterations in humidity may cause no alterations in water loss from the skin, except in so far as capillary pressure is affected by circulatory adjustments (Bazett, Phelps, Adolph).

Sweat gland activity is an emergency process which comes into play in warm environments, omitting psychic disturbances and the like. The output of sweat is controlled by temperature. With unlimited perspiration, the amount evaporated depends upon vapor pressure and air movement.

From this it is clear that in ordinary conditions the rate of heat loss from the body and the degree of comfort experienced depend largely upon the combined effects of radiation and convection.

The ordinary thermometer fails to give an indication of these effects because it is not sufficiently affected by radiation and little if any by air movement. A room may feel too cool at 75° or too warm at 60°, depending upon these radiation convection effects.

The effective temperature index also fails in this respect because it does not allow for radiation effects; it overestimates the influence of humidity at ordinary room temperatures; and it

underestimates the cooling effect of low air velocities.

The dry Kata thermometer is oversensitive to air movement while the wet Kata is oversensitive to both wet-bulb temperature and air movement.

Various instruments have been devised to register these radiation convection effects for the purpose of ascertaining the relative comfort of various environments and for studying the effectiveness of different methods of heating and ventilation. The principal ones are (a) Dufton's eupatheoscope,[7] (b) Vernon's globe thermometer,[8] (c) Winslow and Greenburg's thermo-integrator,[3, 4] and (d) Yaglou's heated globe.[5]

The eupatheoscope is an elaborate laboratory instrument designed especially for conditions prevailing in England. Modification is necessary for use in hot weather. It is not entirely suitable for routine measurements in the field, and the cost is quite high.

Vernon's globe thermometer is the simplest of all. It consists of a 6″ blackened copper sphere with an ordinary thermometer in the center. It is best suited to rooms heated by radiant methods. Its readings are sometimes misleading in rooms heated by convection methods. For instance, if the surrounding walls and glass are below air temperature, the globe will indicate in still air a temperature below that of the air, but if the air is set in motion the globe temperature will increase, approaching the temperature of the air as the velocity increases. Thus the cooler of the two conditions (that with high air movement) would give the higher reading on Vernon's globe. When the air and surrounding surfaces are at the same temperature, Vernon's globe records air temperature no matter what the velocity may be.

The thermo-integrator is a decided improvement over the eupatheostat with respect to both principle and operation. It consists of a blackened hollow copper cylinder 24″ long and 8″ in diameter, with hemispherical ends, containing an electric heating element of 21.5 watts (a storage battery furnishes the current). This is equivalent to a heat dissipation by the instrument of 17.5 B.t.u. per square foot per hour. Eight iron-constantan thermo-couples are distributed over the surface of the instrument, and the mean surface temperature is recorded automatically. This gives a valuable index of the radiation-convection condition of the environment to which the instrument is exposed.

The heated globe thermometer consists of 2 or 3 hollow spheres, one inside another, with air spaces in between to simulate the air spaces in between the clothing of a man. The outer sphere is a blackened standard 6″ tank float. The innermost sphere contains a heating element of variable resistance suitable for connection to the ordinary lighting circuit. The heat input is kept constant for any particular scale, but the amount can be varied by adjusting the resistance from a minimum of 15.3 B.t.u. per sq. ft. per hr. for men at rest, to a maximum of 75 B.t.u. or more for men at work, thus giving a variety of scales for various rates of work. During the heating season the electrical input is at the rate of 15.3 B.t.u./sq. ft./hr. for men at rest, and the surface of the outer sphere is kept dry. In summer the heat input is increased to 20.5 B.t.u./sq. ft./hr. and the lower half of the globe is moistened by fitting on a knitted woolen cap with a tassel which dips into a small bottle of water. When physical work is done at a rate which induces general sweating, the whole surface of the globe must be covered with a wet cloth and the electrical input increased to correspond with the total metabolism. An ordinary glass thermometer inside the outer

air space of the instrument registers a temperature comparable to that existing in the human body between the two outer layers of clothing. The readings of such a globe thermometer seem to be closely related to sensations of warmth and comfort induced by various air conditions.

A single 6″ heated globe with a thermometer close to the inner surface of the wall is as good as a triple globe for physical records of the intensity of radiation and convection. A cadmium plated cylindrical heater ⅜″ in diameter by 1½″ long, slipped over the bulb of an ordinary glass thermometer, is used as an anemometer by means of which air movement may be determined within ±¼ ft. per min.

How valuable the thermo-integrator or heated globe may be as a measure of atmospheric comfort remains to be seen. All four instruments apply during the heating season when the usual changes of humidity exert but a minor rôle upon comfort and sensations of warmth. Under conditions of sensible perspiration it would be desirable to retain the effective temperature index, at least for the time being, for it is difficult to reproduce the surface wetness of clothing and exposed skin by the addition of a moist covering to any of the four devices described.

DESIRABLE AIR CONDITIONS

The test for adequate air conditions should be the comfort of the individual, his psychological and physiological reactions to the conditions, and, last but not least, his inclination for work. No single comfort standard can be fixed to meet every purpose. The requirements differ according to the state of health, sex, age, clothing, activity, and degree of acquired adaptation.

For persons at rest or in sedentary occupations, the air conditions shown by the comfort zones of the A.S.H.V.E.[9] will generally prove satisfactory, provided a correction is made for radiation-convection effects, whenever necessary, by the use of the thermo-integrator or the heated globe thermometer. Such a correction may be made by using the radiation-convection temperature shown by the heated globe thermometer instead of the dry bulb temperature in computing the effective temperature.

For men at work definite standards for physical air factors are almost impossible to establish owing to the chang-

TABLE I

METABOLIC RATES OF MEN IN VARIOUS TRADES AND PROBABLE EFFECTIVE TEMPERATURE REQUIRED FOR COMFORT

	B.t.u. per Hour	*Authority*	*Probable Desirable Effective Temperature for Comfort* [10, 11, 12]
Seated, at rest	384	A.S.H.V.E.	*66* *
Tailor	482		65
Bookbinder	626		64
Shoemaker	661		63
Carpenter	762–963	Becker and Hamalainen	62–58
Metal worker	862		59
Painter (of furniture)	876		59
Man sawing wood	1,797		42
Maximum exertion	3,333 to 4,762	Henderson and Haggard	?

* Value holds for comfort during the heating season. In summer the probable comfortable E.T. varies from 68 to 75 E.T. depending upon prevailing outdoor temperature and conditions of exposure.

ing character of the requirements, particularly with respect to the type and rate of work, type of occupancy, duration of exposure, clothing, adaptation, etc. Each industrial process presents its own factors. The manufacturer should study and regulate conditions so as to bring about maximum comfort and work output.

A suggested schedule of desirable air conditions in various trades is given in Table I on the basis of total metabolism during work. The values are rough estimates indicative only of the extent of the variation in the requirements of industrial workers.

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Chemical Procedures in Air Analysis
Methods for Determination of Poisonous Atmospheric Contaminants

WARREN A. COOK

THERE is at this time no central source of information readily available on methods of determination of air-borne poisons. The only treatise on this subject appears to be a Russian publication of 188 pages, *Methods for the Determination of Obnoxious Gases and Vapors in the Air*, by A. S. Zhitkova, which appeared in 1934. An English translation has been made of this but it has not yet been made generally available. Although its bibliography refers, with a few exceptions, to Russian authors, and the author had apparently little access to recent American literature, this publication has a definite value.

Information on the determination of obnoxious materials found in the atmosphere is scattered throughout a variety of books, journals, and reports. Many of the published methods do not include collection of the material from the air nor discussion of the application of the methods to specific conditions.

A comprehensive work on the subject of determination of poisonous atmospheric contaminants may well be undertaken, it would seem, by the standing Committee on Ventilation and Atmospheric Pollution whose reports should present the consensus of opinion of those actively engaged in the field. Approach to this task by a sub-committee appointed for the purpose seems therefore especially desirable. The subject is dynamic, papers being constantly published on new or improved methods. At this time when great interest is being evinced in this field, it also seems desirable to correlate the available methods to the end that more reliable work can be done, that results of various interested investigators may be comparable and that re-duplication of effort in the development of new methods may be avoided.

Such a sub-committee's work may well be directed along the following lines:

1. The preparation of a list of the more common poisonous atmospheric contaminants together with satisfactory methods for the determination of them in the air, and original references to these methods
2. The preparation of a list of the toxic thresholds of these materials under varying conditions of time and concentration
3. The preparation of a list of those injurious materials for the determination of which new or improved methods should be developed
4. The preparation of an annual annotated bibliography of the literature on the subject
5. The collection of information from those actually using the various methods to ascertain their reported reliability for various applications
6. The affording of a means of exchange of information and experience among those working in the field
7. The stimulation of further research in the whole field on a broader and firmer basis

The present report is limited to a brief discussion of the first item in the above outline, viz., methods for the determination of the more common inorganic toxic materials to which workers are exposed.

METHODS AVAILABLE FOR THE DETERMINATION OF POISONOUS ATMOSPHERIC CONTAMINANTS

The types of methods which are of greatest utility are those which permit obtaining the result at the time and place of the determination without recourse to the laboratory. Methods of this type are principally those based on physical and physico-chemical phenomena. Those which have the widest use are (1) the portable interferometer, (2) combustion apparatus, usually depending upon catalytic combustion, (3) adsorption on solid materials such as activated charcoal and silica gel, (4) apparatus dependent upon the thermal conductivity principal, (5) methods based on refractive index, (6) micro-chemical methods, (7) spectroscopic methods, and (8) electroscopic methods.

Most of the above methods are applied to the determination of gases and vapors in air. Portable equipment based on the principles of interference of light, catalytic combustion, and thermal conductivity are now manufactured and may be applied to a wide variety of vapors and gases. While further developmental work should be conducted on certain of the applications of these instruments, these and other physico-chemical appliances possess the advantages of being less laborious than chemical methods, of frequently permitting immediately available results, and of enabling the plant engineer himself, in many instances, to obtain the desired information. The major disadvantage of most of the above methods is that they are not selective for any one of a mixture of gases or vapors but show the sum of all present. However, the information concerning the total concentration of a gas or vapor is often sufficient to serve as a basis for judgment of the presence and extent of the health hazard presented. Where more specific information is required, as to the concentrations of one of several gases or vapors, chemical methods must usually be employed. Chemical methods are also more generally applicable to solid materials in the air.

CHEMICAL METHODS

The determination of injurious materials in air by means of chemical methods usually involves three steps: (1) A satisfactory method of collection of the material, (2) the separation of the material from interfering substances, and (3) an appropriate procedure for determining extremely minute quantities.

COLLECTION OF THE SAMPLE

Since the amount of the injurious material in the air is usually very small, the method of collection of the sample usually involves passing a large volume of air through an apparatus which retains the material to be analyzed. Since the impinger apparatus (described by Bloomfield in another section of this report) samples air at the high rate of 1 cu. ft. per min., this device is of value where it is shown that it collects the injurious materials with the necessary efficiency. Even where gas sampling bottles of the most efficient types are used, it is desirable to conduct laboratory tests to determine their efficiency at a given sampling rate before using them in the field. This caution may also be suggested in the use of all methods for determining atmospheric constituents.

METHODS OF DETERMINATION

A list of the more common inorganic atmospheric contaminants is appended together with references to methods for their determination in air. Many of these methods have been actually used for making field determinations and have been found to be satisfactory by the writer; others of the methods are

suggested in authoritative literature and are believed to be reliable. It is urged that the use of these methods be subjected to laboratory tests for a given situation before employing them. In due time this committee aspires to give the most satisfactory methods a formal approval.

Material	*Method of Collection*	*Method of Determination*
Oxygen	Take sample of air in gas collecting pipette.	Absorb oxygen in Fieser's solution (alkaline hyposulphite with anthra-quinone-betasulphonic acid). Reference: *J. Am. Chem. Soc.*, 46:2639, 1924.
Ozone	Pass air through alkaline potassium iodide solution in gas wash bottles.	Acidify with sulphuric acid and titrate the liberated iodine with 0.01 normal thiosulphate solution. This method specific only in the absence of chlorine. Reference: Flury, F., and Zernik, F. *Schädliche Gase, Dämpfe, Nebel, Rauch- und Staubarten.* Julius Springer: Berlin, 1931, p. 116.
Chlorine	Pass air through o-tolidine reagent in absorption tube described by Porter.	Transfer sample to Nessler tube and compare color with standards after standing for at least 2 minutes. Reference: Porter. *J. Indust. & Eng. Chem.*, 18:730, 1926.
Bromine	Pass air through potassium hydroxide solution in bubbler.	Determine the bromide as silver bromide. Reference: Flury and Zernik. *Schädliche Gase*, 1931, p. 123.
Iodine	Pass air through freshly prepared 10% potassium iodide solution in bubbler.	Titrate with standard thiosulphate solution. Reference: Flury and Zernik. *Schädliche Gase*, 1931, p. 124.
Hydrochloric Acid	Pass air through glycerol-potassium carbonate-water solution of the ratio 1:1:1.	The chloride ion is determined by the Volhard method. Reference: Heller, A. Determination of Hydrochloric Acid in the Air. *Gesundheits-Ingenieur*, 55:261, 1932. *Chem. Abst.*, 27:155 (Jan. 10), 1933.
Hydrofluoric Acid	Pass air through gas wash bottles containing potassium hydroxide solution.	Distil fluorides as hydrofluosilicic acid. Determine fluorides in distillate by titration with thorium nitrate solution using sodium alizarine sulphonate as the indicator. Reference: Willard and Winter. *Indust. Eng. Chem.*, Anal. Ed., 5:7, 1933; Boruff and Abbott, *Ibid.*, 5:236, 1933.
Sulphur Dioxide	Pass air through gas wash bottles containing measured volume of standard iodine solution in potassium iodide to which starch solution has been added. Stop sampling before purple color is entirely removed.	Titrate remaining iodine with standard thiosulphate solution. Reference: Adaptation from Fieldner *et al. J. Indust. & Eng. Chem.*, 11:519, 1919.

Material	*Method of Collection*	*Method of Determination*
Sulphur Dioxide (Cont.)		A detailed account of the iodimetric method given in following reference: Griffin and Skinner: Small Amounts of Sulfur Dioxide in the Atmosphere I. Improved Method for Determination of Sulfur Dioxide When Present in Low Concentration in Air. *Indust. Eng. Chem.*, 24:862, 1932.
	Aspirate the air through an absorber containing hydrogen peroxide and sulphuric acid in an automatic apparatus.	Analysis of samples which are automatically transferred to sample bottles by means of the iodimetric method. Reference: Thomas and Cross. Automatic Apparatus for the Determination of Small Concentrations of Sulfur Dioxide in Air. *Indust. Eng. Chem.*, 20:645, 1928. Thomas and Abersold. Part II of above. *Ibid.*, Anal. Ed., 1:14, 1929. Thomas. Part III of above. *Ibid.*, 4:253, 1932.
Hydrogen Sulphide		
	Aspirate air through a glass tube containing sensitized granules of activated alumina coated with silver cyanide or lead acetate.	The concentration is indicated by the length of travel of the color change on the granules with a given volume of air sampled or by the volume of air required to produce a standard length of travel. Reference: Littlefield, Yant, Berger. A Detector for Quantitative Determinations of Low Concentrations of Hydrogen Sulfide. U. S. Bureau of Mines, *Report of Investigations No. 3276*, June, 1935.
	Collect sample as given under iodimetric method for sulphur dioxide.	Determine hydrogen sulphide according to method given by Griffin and Skinner for sulphur dioxide.
Sulphuric Acid		
	Pass air through gas sampling bottle containing sodium hydroxide solution.	Acidify sample with hydrochloric acid and precipitate sulphates with barium chloride.
Sulphur Chloride		
	Conduct air slowly through a gas washing bottle containing a nitric acid solution of silver nitrate.	Dissolve the precipitated silver chloride in ammonia and again precipitate with nitric acid. Reference: Flury and Zernik. *Schädliche Gase*, 1931, p. 147.
Ammonia		
	Pass air through sulphuric acid solution in gas wash bottle.	Determine ammonia by A.P.H.A. Standard Method. Reference: A.P.H.A. *Standard Methods for the Examination of Water and Sewage.* 7th ed., 1933.
Nitrous Oxide		
	Pass air through sodium hydroxide solution in gas washing bottle.	Determine as nitrite in accordance with A.P.H.A. Standard Method. Reference: *Ibid.*
Nitrogen Oxides (Mixture of various oxides of nitrogen, principally NO_2 and N_2O_4)		
	Pass air through sodium hydroxide solution in gas wash bottle.	Oxidize with hydrogen peroxide and determine nitrates by A.P.H.A. Standard Method. Reference: *Ibid.*
Nitric Acid		
	Pass air through sodium hydroxide solution in gas wash bottle.	Method of determination similar to above.

Material	*Method of Collection*	*Method of Determination*
Phosphine	Pass air through gas washing bottle containing bromine water or through solution of sodium hypochlorite or bromite.	Expel the bromine. Determine as phosphoric acid. Reference: Flury and Zernik. *Schädliche Gase*, 1931, p. 171.
Phosphorus Trichloride Phosphorus Pentachloride Phosphorus Oxychloride	Pass air through 20% sodium hydroxide solution in gas wash bottle.	Determination of the dissociated chlorine (inexact on account of hydrolysis in the air). Reference: Flury and Zernik. *Schädliche Gase*, 1931, p. 172.
Arsine	Pass the air through silver nitrate solution in gas washing bottle.	Titrate the resulting arsenious acids. Reference: *Ibid.*, p. 179. Other methods of analysis are critically discussed by Joachimoglu, *Arch. f. Exper. Pathol. u. Pharmakol.*, 85:32, 1919.
Carbon Monoxide	The method of choice for low concentrations is that depending upon the combustion method in which Hopcalite is used as the catalyst. The concentration of carbon monoxide is read directly from the dial of a milliammeter calibrated to read in parts per million of carbon monoxide. For higher concentrations the "Hoolamite" detector depending upon the color change produced by liberation of iodine from iodine pentoxide is a convenient method. Collection of sample of air in a gas pipette.	The sample of air is passed through a tube containing iodine pentoxide at 300° C. and the liberated iodine is passed into potassium iodide solution. The iodine is determined by titration with standard thiosulphate solution. Reference: The many methods and references on carbon monoxide determination can be completely handled only by a more extensive treatise.
Carbon Dioxide	Pass air into gas washing bottle containing dilute barium hydroxide solution.	Titrate with dilute hydrochloric acid or oxalic acid using phenolphthalein as an indicator. References: Fieldner *et al.* *Indust. Eng. Chem.*, 11:519, 1919.
Phosgene	Air is passed through gas washing bottles containing 50 c.c. alcoholic sodium hydroxide, prepared by dissolving 40 g. sodium hydroxide in 125 ml. of distilled water and adding the solution to 875 ml. of 95% ethyl alcohol.	Neutralize with nitric acid (approximately normal), using phenolphthalein as an indicator, and the chloride ion is determined by titration with 0.1286 *N* silver nitrate using sodium chromate as an indicator. 1 ml. of 0.1286 *N* silver nitrate equals 1,000 p.p.m. phosgene (by volume at 25° C. and 760 mm.) for a 1 minute sample. Reference: *Ibid.*, p. 523.
Cadmium or Cadmium Compounds	Impinger device using water as the liquid.	Precipitate the cadmium as the sulphate and determine gravimetrically. Reference: Treadwell-Hall. *Quantitative Analysis.* Precipitate with hydrogen sulphide, dissolve, remove acid by evaporation, treat with KCN solution and some H_2S water. The color of the precipitate is then compared with that of similarly treated standard solutions under ultra-violet light.

Material	*Method of Collection*	*Method of Determination*
Cadmium or Cadmium Compounds (Cont.)		Reference: Fairhall and Prodan. Colorimetric Determination of Minute Amounts of Cadmium. *J. Am. Chem. Soc.*, 53:1321, 1931.
Mercury	Mercury vapor may be determined by means of the Nordlander Apparatus in which air at 70° C. is impinged against paper coated with selenium sulphide (dry). The concentration of mercury vapor is determined by comparing the degree of darkening of the yellow selenium sulphide with that of a standard paper.	Reference: *Indust. Eng. Chem.*, 19:522, 1927.
	Passing air through liquid air trap for condensation and collection of mercury.	Electrolytic deposition of mercury. Final estimation of amount of mercury recovered by measuring size of mercury sphere by means of the microscope.
	Absorption of mercuric bromide, produced by reaction of mercury vapor with bromine, in water.	Reference: Stock and Cucuel. Determination of Mercury Content in Air. *Ber. d. deutsch. chem. Gesellsch.*, 67:122, 1934.
	The above methods were developed for the determination of mercury vapor.	
	The vapor is collected by a freezing method using solid carbon dioxide.	Electro deposition of the mercury and micrometric determination similar to that described by Stock.
		Reference: Fraser. The Determination of Mercury in Air and in Urine. *J. Indust. Hyg.*, 16:67, 1934.
Lead	Impinger device with either water or dilute nitric acid as the liquid.	Precipitation of the lead as the sulphide and final estimation as the chromate.
		Reference: Fairhall: Lead Studies I. The Estimation of Minute Amounts of Lead in Biological Materials. *Ibid.*, 4:9, 1922–23.
		Precipitation of lead as the sulphide and final estimation as the chromate.
		Reference: Kehoe, Thamann, and Cholak. On the Normal Absorption and Excretion of lead. I. Lead Absorption and Excretion in Primitive Life. *Ibid.*, 15:257, 1933.
		Reference: Cholak. Quantitative Spectrographic Determination of Lead in Biological Material. *J. Indust. & Eng. Chem.*, Anal. Ed., 7, 5:287–290 (Sept. 15), 1935.
Chromic Acid	Impinger device with approximately normal sodium hydroxide as the liquid.	Determination of the chromate iodimetrically.
		Reference: Bloomfield and Blum. Health Hazards in Chromium Plating. *Pub. Health Rep.*, 43: 2330, 1928.
		The chromate may be determined with semi-diphenylcarbazide as the indicator.
		Reference: Cazeneuve, P. *J. de pharm. et de chim.*, 12:150, 1900; abstr. in *Analyst*, 25:331, 1900.
Radioactive Substances	Methods of determination of radioactive substances in the air together with references supporting these methods are given in the following papers describing an extensive research conducted by the U. S. Public Health Service.	References: Schwartz, Knowles, Britten and Thompson. Health Aspects of Radium Dial Painting I. Scope and Findings. *J. Indust. Hyg.*, 15:362, 1933. Bloomfield and Knowles. II. Occupational Environment. *Ibid.*, p. 368. Ives, Knowles, Britten. III. Measurements of Radioactivity in Workers. *Ibid.*, p. 433.

Dust Procedures in Air Analysis

The Sampling and Analysis of Industrial Dusts

J. J. BLOOMFIELD

THE subject of the health of workers in dusty trades has been receiving considerable attention from students of industrial hygiene and others interested in the various phases of this problem. When one realizes that the workmen employed in the dusty trades comprise one of the largest groups exposed to any one industrial hazard, it is quite apparent that the importance of this problem has not been overestimated. Furthermore, it is now well established that exposure to certain kinds of dusts, such as those containing considerable amounts of quartz, has increased the morbidity and mortality rate from respiratory disease; while metallic dusts, such as lead and its compounds, have been associated with general systemic poisoning. Thus, the harmful properties are the composition of the dust, the quantity suspended in the industrial atmosphere, and its particle size.

Investigations of the industrial dust question serve a threefold purpose. First, they enable one to evaluate the extent of the hazard; this is accomplished by obtaining occupational dust exposures, which disclose the dust creating tasks. Second, if clinical studies are also made, dust counts may indicate the permissible amount of dust which may be breathed with impunity. Third, dust determinations are used in an attempt to control the hazard; this is effected by testing the efficiency of devices which have been developed for this purpose.

The quantitative aspects of the dust problem demand standardized procedure in the sampling and analysis of dusts. The American Public Health Association, through its Committee on Standard Methods for the Examination of the Air, made its last report October 27, 1919. This was published in May, 1920.[1] Here it recommended the continuation of the Palmer Water Spray Apparatus as the standard method of dust determination and suggested that if the konimeter[2] or Bill electrostatic collector,[3] developed at that time, proved to be superior, then one or the other of these devices should be adopted.

In 1922, the instruments in common use in conducting dust studies were the Palmer, konimeter, and sugar tube, and, in addition, the U. S. Bureau of Chemistry,[4] in its dust explosion work, employed an apparatus which consisted essentially of an adapter for holding a Whatman filter paper thimble through which air was drawn by a suction pump. By the difference in weight of the paper thimble before and after dust sampling, the weight of the dust was easily determined. Finally, mention should be made of the Anderson and Armspach[5] dust determinator which in 1922 was in use by the American Society of Heating and Ventilating Engineers. This instrument measured the increase of pressure incident to forcing air through a piece of filter paper; this rate of increase of pressure was then taken as a measure of the air dustiness.

However, in the year 1922, it became apparent that the various methods did not yield results which could be

regarded as absolute or even comparable. Consequently, a conference of interested persons was held at the U. S. Bureau of Mines Experiment Station, Pittsburgh, Pa., in 1922, when it was decided to conduct a laboratory study of dust sampling instruments.

Suspensions of dust (5 different powdered substances) were set up in an air-tight chamber at the Experiment Station that year, and simultaneous samplings were carried out with the sugar tube, the Palmer apparatus, the konimeter, the filter paper thimble, and the dust determinator.

During the course of the study, a new instrument, the Greenburg-Smith impinger, for the sampling of dust, was devised (see *Pub. Health Bull. No. 144*). In this instrument, the air to be sampled is drawn through a glass tube and impinged at a high velocity on a glass plate which is kept beneath the surface of the water or other suitable fluid in the collecting flask. The dust is momentarily arrested, wetted by the collecting fluid, and in this manner trapped. After a sufficient volume of air has been sampled, a portion of the collecting fluid is removed to a suitable counting chamber, or cell, for microscopic count, to ascertain the number of particles. The remaining portion of the sample may be subjected to any desired analysis.

In the comparative study, the dust catching efficiency of the impinger was found to be high. Consequently, its physical principles and characteristics were the object of a special study, and finally a satisfactory and practical form of dust sampling instrument, based on this principle, was evolved.*

The apparatus (essentially in its present form), as described in *Public Health Bulletin No. 144*, possessed an efficiency of 94 to 97.5 per cent when sampling a finely divided silica dust suspension at the rate of 1 cu. ft. per minute. The tests used in estimating this efficiency were conducted by an optical method in which a portion of the dusty air delivered to the collecting device was diluted with measured amounts of dust-free air until a "match" was obtained on comparison with the stream of air emerging from the dust collecting device. The comparison, or matching, consisted in producing equal Tyndall effects (equal amounts of reflected light) by the two dust streams when they are simultaneously observed in a beam of light.

So far as the quantitative results of the dust sampling instruments are concerned, the conclusion of the comparative study was as follows:

> Considering the dust caught by the Palmer as unity, the instruments take the following order: On basis of numbers of particles determined—impinger, 5.0; sugar tube, 2.1; and Palmer apparatus, 1.0. On basis of weight of dust determined—impinger, 2.1; thimble, 1.5; sugar tube, 1.6; Palmer apparatus, 1.0.

These results, as well as others obtained in a comparative field study of the Palmer, konimeter, and impinger,[6] led to the selection of the impinger (Greenburg-Smith) by the Public Health Service as the standard device for dust sampling.

Several other instruments for the collection of dust have been developed since 1922. These are the Owens jet dust counter,[7] Drinker's modification of the electrical precipitator device,[8] and, more recently, Green has described a sedimentation cell method[9] and a thermal precipitator device,[10] which are being tried out in England. Of these four devices, the Owens and the electrical precipitator are the only ones

* The instruments and methods described in this paper are generally used in conducting research investigations for the purpose of evaluating the dust hazard in industry. Some are of the opinion that other instruments or methods of quantification of dust may be used for routine plant control of a dust hazard, provided of course that these simpler and quicker methods have been correlated with the standard procedure described in this paper.

which have been used to any great extent and for which data on performance are available. It may be safely stated that in so far as *present day practice* goes, the instruments finding common use for dust collection throughout the world are the impinger, konimeter, Owens, electrical precipitator, and the paper thimble. The others have either been discarded, as in the case of the Palmer and Anderson-Armspach dust determinator, or are still in the developmental stage, as is true of the sedimentation cell, thermal precipitator, and more recently, the electrostatic precipitator being developed by the Westinghouse research workers.*

THE SAMPLING OF INDUSTRIAL DUSTS

General Considerations—It is essential that any dust sampling instrument should be capable of collecting effectively dust of a size present in the industrial atmosphere. Recent studies by the author [11] indicate that industrial dusts are mainly from 0.5 to 5 microns in size, so that an instrument capable of collecting these sizes with a fairly high degree of efficiency should meet the present-day requirements. The range in dust concentrations encountered in industries is very great, depending, of course, on the industrial processes, the devices installed for mitigating the dust created, and the efficiency with which such devices function. For these reasons, an ideal instrument should be one capable of sampling with equal efficiency in both high and low dust concentrations. This factor calls for the prerequisite of a dust collecting medium which shall not add dust to any great degree to the dust in the sampled air. Furthermore, the collecting medium must be uniform in dust content, so that one or two control tests on this medium will be sufficient for a series of samples.

Since the dust content of industrial air is ever varying, it is essential that the dust sampling instrument be capable of collecting air in large quantities at a rapid rate, in order to obtain a representative picture of existing conditions. And, finally, the ideal instrument should be one that requires only a simple and fairly rapid method of analysis. Suffice it to say that an instrument designed for use in the field should embody all these principles, and, in addition, be light in weight, portable, and compact.

To recapitulate, the final selection of a dust sampling instrument for industrial use will depend on the collecting efficiency of the device, on its small errors in analysis, on its portability, and on the ease with which a sample, once obtained, may be analyzed.

Twelve years of experience with the impinger apparatus in this country by the U. S. Public Health Service,[12] by various research workers in our universities, insurance laboratories, and other interested governmental and industrial agencies, have shown this instrument to be of practical value as a device for the study of the industrial dust problem and one apparently filling the requirements set forth in the above discussion. Although the other devices, such as the konimeter, Owens, electrical precipitator, and paper thimble, are utilized from time to time in special problems, the impinger has been adopted quite universally in this country in hygienic dust investigations. For this reason a brief account of the construction and use of this device is presented herein. For details of the other instruments mentioned in this discussion the reader is referred to the original articles describing each, references to which have been given.

The Impinger Tube and Sampling Flask—The impinger apparatus consists essentially of two portions: First, a source of suf-

* Personal communication.

ficient suction to draw the air to be sampled through the sampling device; and second, the sampling device or impinger itself, which consists of a container and the impinger tube and plate. As a source of suction, an electrically driven and a compressed air driven apparatus have been designed. A hand driven apparatus developed at the U. S. Bureau of Mines has also been made. For details of construction of these various forms of suction devices the reader is referred to the original article.[12]

The impinger tube in the model of the apparatus described in *Public Health Bulletin No. 144* (p. 67) consisted of a piece of Pyrex glass tubing, drawn down to a tip with a 2.3 mm. orifice. To this tube a metal tripod and circular impinging plate were attached by means of a bronze split-sleeve clamp. The distance between the orifice and the upper surface of the plate was kept at 5 mm.

In practice, this impinger tube yielded satisfactory results. Nevertheless, it was felt that it would be preferable to eliminate the use of metal, particularly where acid or alkali was to be used as the collecting fluid. Accordingly, there was designed and constructed the all-glass impinger tube shown in Figure I, with circular glass impinger plate about 25 mm. in diameter, fixed by three

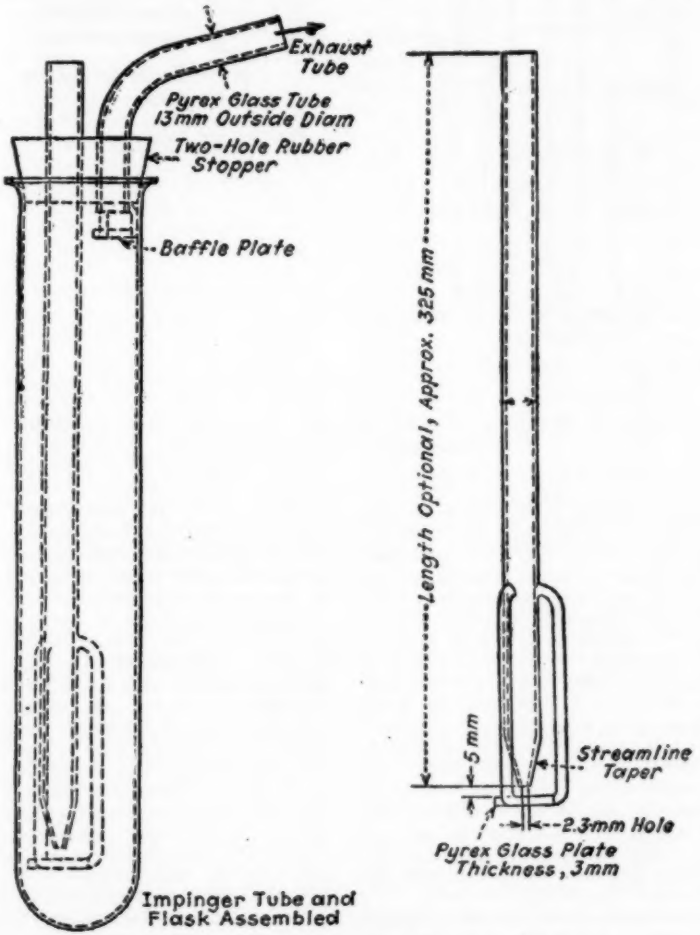

FIGURE I—DRAWING OF IMPINGER TUBE AND FLASK

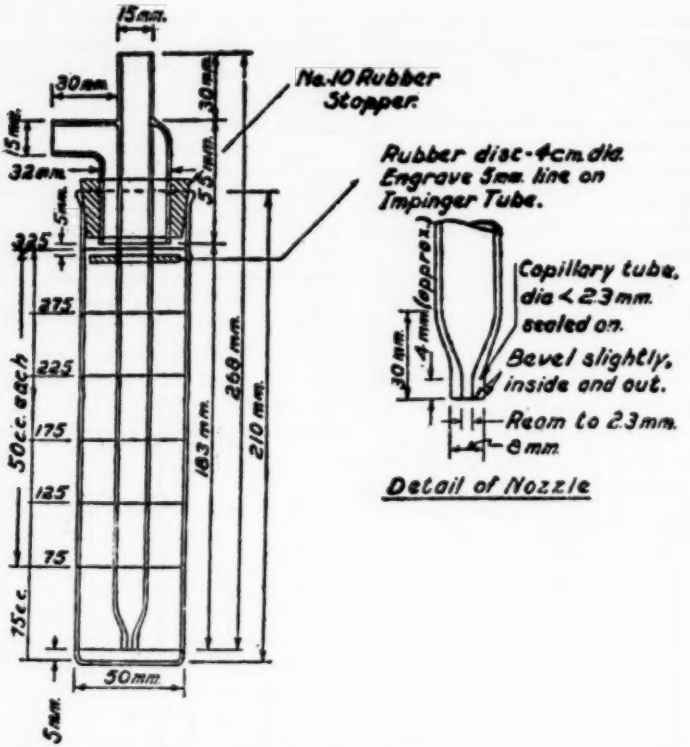

FIGURE II—DRAWING OF MODIFIED IMPINGER AND DETAIL OF NOZZLE, WITH CONSTRUCTION SPECIFICATIONS

supporting rods about 9 cm. long to the impinger tube at a distance of 5 mm. from the orifice. The tube was 13 mm. in outside diameter. Pyrex glass was used throughout. Tubes of this type have been employed without an undue amount of breakage.

A few modifications have been made in the impinger flask. Originally, a round 16 oz. glass bottle fitted with a 2 hole rubber stopper was used. A short time later a 500 c.c. Pyrex glass assay flask (wide-mouth conical Erlenmeyer type) was substituted, which proved to be very satisfactory.

In certain studies it was found desirable to obtain dust samples in the region of the mouth and nose of a worker in order to secure a more representative picture of the air actually breathed. For this purpose the flask shown in Figure I was designed.

A more compact and convenient impinger flask and nozzle has been recently developed for field use.[12a] The modifications in the design of the impinger have in no way altered the basic principle on which the instrument operates. Figure II shows the latest form of the impinger flask and nozzle.

The modified impinger does away with the glass impinging plate, utilizing the bottom of the flask for this purpose. The suction connection is combined with the inlet tube, thus simplifying the device still further. The essential portions of the device consist of a straight piece of Pyrex glass tubing 15 mm. in outside diameter and approximately 275 mm. in length. The tube is drawn down in stream-line form at its lower end, to a tip with a 2.3 mm. orifice. In sampling, this orifice is kept approximately 5 mm. from the bottom of the flask, a guide line on the flask indicating this distance. The flask is 50 mm. in diameter and 210 mm. in height, and requires a fluid (water) volume of only

75 c.c. to give the proper depth of immersion to the nozzle. An entrainment trap in the form of a rubber ring prevents the possible loss of the liquid (and dust) with the outgoing air.

Since the impingement distance in this instrument is not fixed, as in the previously described type, it was necessary to determine the limits within which this distance, as well as the angle of impingement from the perpendicular, varied. Studies conducted on these factors by the designers of this instrument showed that as regards the angle of impingement (using silica dust) one can have a maximum displacement of the nozzle without changing the efficiency obtained when the nozzle is in the vertical position. Also, it was found that the impinging distance can be varied from 2 to 12 mm. without impairing the efficiency against silica dust to any appreciable degree.

In taking dust samples the location of the sampling place, the time during which sampling is conducted, and the duration of sampling, are all chosen in an effort to obtain the data required by the study in progress. Obviously the requirements of the study under way govern the procedure to be employed.

The duration of the sampling period should be such as to yield a satisfactory suspension of dust for analysis, and is thus dependent on the concentration of dust in the atmosphere. Under the usual industrial conditions, samples of from 10 to 30 cu. ft. of air are sufficient to yield enough suspended dust for analysis. Since a sampling rate of 1 cu. ft. per minute is maintained, this will require a sampling period of from 10 to 30 minutes. A stop watch may be used to measure this period. After a sample is obtained, the impinger tube and flask are placed in a suitable carrying case for transport to the laboratory for subsequent analysis.

In Table I, a summary is presented giving the characteristics of the most commonly used dust sampling instruments.

It may be seen that all of the devices have a high sampling efficiency. Although each instrument has some disadvantages, experience has shown that there is a specific use for each device. The *impinger apparatus* can be employed for sampling in both high and low dust concentrations, and has the added advantage that the samples may usually be analyzed microscopically, chemically, or gravimetrically. In fact, it has also proved useful for sampling fumes and gases, provided certain precautions are exercised.[13, 14] The *electrical precipitator* finds its greatest field in the sampling of smoke and fumes. The *paper thimble* has been used very successfully for the sampling of radioactive dusts,[15] and is constantly employed in sampling organic dusts in connection with dust explosion prevention work. The *Owens jet dust counter* was primarily developed for the sampling of outdoor dusts and smoke, and is still used quite universally for this purpose. It is usually not practical for the sampling of industrial dusts of high concentration, the dust record being so thick as to make it impossible of enumeration. However, it has been used successfully for obtaining samples of industrial dusts for the purpose of particle size measurements.[11] The *konimeter* is very useful in obtaining rapid samples in moderate concentrations of aerial dusts. For quantities of 15 to 18 million particles or less per cu. ft. of air, it is very efficient and should find considerable use in control work and in preliminary studies. For such investigations the *Owens* and *konimeter* have the added advantage that they require no power for operation, are very small and compact, and do not need a highly skilled observer to obtain samples, although some skill is required in the enumeration of the dust records and in the interpretation of results.

Where the properties of an industrial dust, both chemical and physical, may vary from hour to hour at a given work position, due to the varying nature of the process, *e.g.*, periods of blasting, hard-heading, uneven ventilation, etc., additional precautions in sampling are obviously necessary.

So far this discussion has been

TABLE I

SUMMARY OF CHARACTERISTICS OF CERTAIN DUST-SAMPLING INSTRUMENTS

Instrument	*Characteristics*								
	Principle of operation	*Efficiency against industrial dusts (per cent)*	*Application*	*Method of quantification*	*Skill in quantification*	*Sampling skill*	*Volume of sample*	*Advantages of Instrument*	*Disadvantages of instrument*
Impinger	Impingement	98+	General	Count, gravimetric, chemical	Considerable	Some	Any amount; rate = 1 cu. ft. per minute	1. High sampling efficiency in either low or high dust concentrations 2. Sample can be quantificated by counting, weighing, or chemical analysis	1. Requires power for operation
Electrical precipitator	Electrical precipitation	100	do	do	do	Considerable	Any amount; rate = 10 to 50 liters a minute	1. High sampling efficiency in either low or high dust concentrations 2. Sample can be quantificated by counting, weighing, or chemical analysis 3. Large samples obtained rapidly	1. Requires electric power for operation 2. Some danger from high voltages
Paper thimble	Filtration	100	do	Gravimetric, chemical	Some	Very little	Any amount; rate 1 to 2 cu. ft. per minute	1. High sampling efficiency 2. Samples large volumes rapidly 3. Laboratory technic requires only drying and weighing for most dusts 4. Samples may be kept indefinitely without deterioration	1. Samples cannot be counted 2. Drying of thimbles is a very slow process
Owens jet dust counter	Jet condensation	99+	Outdoor dust and for particle-size studies	Count	do	do	50 to 1,000 c.c.	1. Light, simple, and quick to operate 2. High efficiency for atmospheric smoke 3. No power needed for operation 4. Laboratory technic requires only a microscope	1. Dust cannot be weighed or analyzed chemically 2. Obtains only "grab" samples due to small sampling volume 3. Impractical in high dust concentrations 4. Selective action; efficient only for dusts 2 microns or less
Konimeter	do	99+	For concentrations less than 18 million particles per cu. ft.	do	do	do	10 c.c	1. Light, simple, and quick to operate 2. Efficiency high for moderate concentrations 3. No power needed for operation 4. Laboratory technic requires only a microscope	1. Dust cannot be weighed or analyzed chemically 2. Obtains only "grab" samples 3. Not practical in high dust concentrations; limited to 15 or 18 million particles, or less, per cu. ft.

limited to the collection of dusts of the type usually considered capable of producing fibrosis of the lungs or upper respiratory damage. For the collection of *poisonous dusts*, such as lead, the impinger or electrical precipitator has been found to be especially suited. In the case of the *impinger*, when used in sampling *lead dusts*, care should be taken that the sampling flask is of lead-free glass and that the distilled water also contains no traces of this substance. It is customary to run a blank for every 10 samples or so, which takes into account any lead present in the reagents. In sampling for minute traces of poisonous compounds, it is very essential that sufficiently large samples be obtained, enough to contain adequate amounts of the material for the particular method of analysis to be employed. It is apparent that both the impinger and electrical precipitator fulfil this requirement owing to their high air sampling rates. While the *paper thimble* has been used successfully for the collection of radioactive dusts, on the whole, the impinger and precipitator collect such dusts in a manner best suited for subsequent chemical analysis.

THE ANALYSIS OF INDUSTRIAL DUSTS

General Considerations—In the case of fibrosis producing dusts, the particle count appears at present to offer the best index of the hazardousness of a dusty operation. Obviously, the method of enumeration which is utilized should be governed by the consideration of the size of the dust which is of hygienic significance. To date, the relative significance of various sizes of dust particles in the production of lung fibrosis has not been satisfactorily established. However, we do know that the inhalation of certain industrial dusts has been found to be associated with definite injury to the pulmonary tissues. Hence, a knowledge of the size-frequency of these industrial dusts will, in a measure, determine the method of enumeration to be employed.

At present there are several sources of information which cast some light on this subject. One is the work of pathologists who have determined the size of dust particles recovered from silicotic lung tissue, another is the work done in connection with the retention of dusts in inhalation experiments, while additional information is presented by particle-size studies.

The work of the South Africans,[16, 17, 18] and Scheid of Germany,[19] indicate that most of the dust particles recovered from silicotic lungs of both humans and animals were between 1 and 3 μ in size, only about 13 per cent of the particles being less than 0.5 μ. Experiments on dust retention by Drinker and his associates,[20, 21, 22] as well as that of other investigators [23, 24] on the retention of dusts and fumes by man, also show that the finely divided suspensions of dust are retained but to a small extent in the lungs, usually from 15 to 25 per cent of the amounts inhaled. More recently, King and Dolan [25] have shown that extremely fine particles of quartz dust are rapidly dissolved in the mildly alkaline body fluids and are excreted in the urine. And finally, particle-size studies of industrial dusts in air [11] indicate that about 60 per cent of the dusts are from 1 to 3 μ in size and that only about 30 per cent of the particles are less than 1 μ.

From the foregoing information it is evident that in order to obtain a representative sample of industrial dust in air, one should employ an instrument capable of arresting, with a high degree of efficiency, all kinds of dusts, of sizes ranging from 0.5 to 5 μ at both high and low dust concentrations. The impinger apparatus fulfils this requirement.

The method of counting the dust particles in the samples should have

small analytical errors and should reveal only those significant particles present in industrial atmospheres. It should not be the aim to count all the dust particles present in the samples (which may be accomplished by either the use of high magnifications, dark-field illumination, or combinations of both), *e.g.*, it is necessary to differentiate between the dust content in normal air and industrial air. As has been indicated, this difference is apparently sharply marked in so far as the dust particles between 0.5 and 5 μ are concerned; but this difference would be masked and lost were there included in the determination the particles of ultra-microscopic size which are present in vast numbers in all air.

Enumeration of Impinger Samples—Owing to the solubility of most industrial dusts in water,[26] it is good practice to analyze impinger samples within 24 hours after collection. Dilution of the sample, if needed, may be made in the original sampling flask, to either 250 or 500 c.c. If further dilution is advisable, a 100 c.c. graduated flask may be employed. This dilution may conveniently be such that the number of particles counted in each microscope field is about 50 to 75. The contents of the flask are next thoroughly agitated in order to obtain a uniform suspension, and two portions of about 1 c.c. each are removed with a pipette so as just to fill, without bubbles, two Sedgwick-Rafter counting cells (see Figure III). The cells have been previously cleaned very carefully in order to remove any adventitious dust, and have been kept protected from dust particles by the cover slip.

In making dust counts an eyepiece micrometer known as a Whipple disk is employed (see Figure III). This disk has a large square engraved on it, covering a large part of the field, and this square is divided into 100 medium sized squares, one of these in turn being further subdivided into 25 very small squares. Using an ordinary microscope provided with a suitable eyepiece and objective, and fitted with an Abbé condenser, the proper tube length of the microscope is determined by calibration with a stage micrometer, so that the side of the large square of the eyepiece covers 1,000 μ (1 mm.). (A 7.5X eyepiece, 16 mm. objective, and a tube length of 178 mm. has been found to yield this result.) The large square of the eyepiece ruling, therefore, incloses the dust in an area of 1 sq. mm.; and since the cell is 1 mm. deep, all the dust suspended in 1 cu. mm. of the water is under the ruled field. This examination is accomplished by raising and lowering the lens system so as to focus throughout the entire depth of the cell. As a source of illumination, an ordinary small electric microscope lamp may be employed. In order to provide a high degree of visibility for refractile objects it is best to lower the Abbé condenser system below the usual focusing point and to restrict the opening in the iris diaphragm.

The dust is allowed to settle for 20 to 30 minutes before counting is done. In general, only particles less than 10 μ in diameter are counted. The inclusion of particles larger than 10 μ in the specimen would make but little change in the total count. The average diameter of a particle for the purpose of this exclusion is judged by inspection. In practice it is necessary to count the dust in only one-quarter of each ruled field, the entire field having been examined for uniformity. Such counts on 5 fields, so dispersed as to be representative, are made on each of the two Sedgwick-Rafter cells. These 10 counts are averaged, but this average is not to be taken as the final count until a corresponding con-

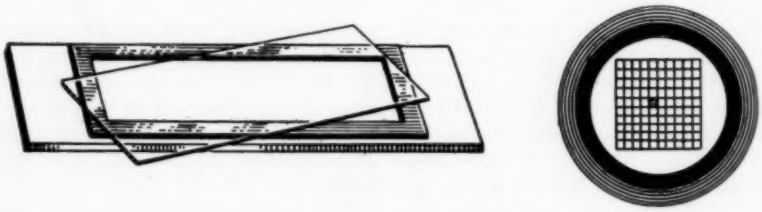

FIGURE III

trol count has been subtracted. In all cases a sampling flask which is handled in the plant, but through which no air has been aspirated, is used as the control for the particular series of samples taken in that plant on that particular day, and counts are made on this control fluid in the same manner as on the fluid through which the air sample has been impinged. The control sample takes into consideration any dust which may be present in the eyepiece micrometer, in the lenses of the microscope, in the Sedgwick-Rafter counting cell, and in the sampling fluid itself. From the average gross count obtained on the impinger sample, the average control count is to be subtracted to give the average net count per ¼-microscopic field.

The average net count per ¼-microscopic field is multiplied by 4 to yield the average count in the total field. Since the Sedgwick-Rafter cell is 1 mm. deep, this figure represents the number of particles in a cu. mm. of the diluted sample. This value is multiplied by 1,000 to give the count per c.c. of sample, and again by the total number of c.c. of fluid to which the original specimen was diluted. This product is divided by the number of cu. ft. of air sampled. In summary, the number of particles per cu. ft. of air = average net count per ¼ field times a factor, where the factor =

$$\frac{4 \times 1{,}000 \times \text{total volume of diluted sample in c.c.}}{\text{Volume of air sampled in cu. ft.}}$$

The method of dust counting presented herein is capable of including dust particles as small as 0.7 μ.[10, 11, 27 *] From data presented earlier on the size-frequency of industrial dusts, it is apparent that this method is capable of disclosing about 85 per cent of the dust particles collected by the impinger apparatus. The small percentage of dust this method fails to reveal is considered negligible, when one takes into consideration the simplicity of the method, the fact that results may be checked by trained observers, and that it is one of practical application since, in the numerous dust studies conducted by the U. S. Public Health Service on the health of workers in dusty trades, high correlations were obtained between the intensity of exposure to dust and the degree of lung damage among the workers.

* Some of the members of the committee are doubtful whether dust particles under 1 μ (and possibly 2 μ) can be seen by means of a light field method of illumination. Hence these resort to a dark field microscopic system almost exclusively, and check this from time to time with the standard light field method.

THE DETERMINATION OF THE CHARACTER AND COMPOSITION OF INDUSTRIAL DUSTS

Size of Dusts—So far as the size of the dust particles is concerned, it is apparent that in order for any given dust to produce injury to the lungs it must gain access to the parenchyma of the lungs, the site where the harmful effects of the dust take place. It has already been shown that not all of the particles of inhaled dust gain access or are retained by the human lungs. For these reasons it is of some value to determine the size of the dust present in the industrial atmosphere.

It has also been demonstrated that particles of a size greater than 10 μ in longest dimension are very seldom found in the lungs, due partly to the fact that the number of such particles, in industrial air, is comparatively small when particles in the lower size range are considered. Due to gravity, which causes rapid settling of suspensions, and due to the protective action of the upper respiratory tract, these larger particles do not penetrate to the terminal portions of the lungs. Hence, it is obvious that attention be directed to those particles which are less than 10 μ in longest dimension.

The likely procedure for the sampling of atmospheric dusts for particle-size studies would be to employ the same instrument utilized in sampling dust for quantitation.

In the past, the Owens jet dust counter has been employed extensively for such investigations, although of late impinger samples have been utilized for this purpose; comparative studies of the two instruments yield practically identical results. The Owens jet dust counter obtains a sample of dust from the air in more or less unaltered con-

dition, since with this device the atmospheric dust is directly projected on a naked coverslip. Moreover, the instrument is small, compact, requires only hand power, and samples are obtained quickly and easily without needing much skill of operation. Once these samples have been obtained and properly mounted, the dust particles may be measured by the use of a filar ocular micrometer at a magnification of 1,000 diameters (oil immersion objective).[28] The horizontal diameter of at least 200 dust particles in several representative fields are measured for each sample. With this magnification it was found possible to measure particles as small as 0.5 μ in size, while particles smaller than this size are easily distinguished at this magnification and their presence recorded.

Photographic methods have been suggested and used for measuring dust particles, but in order to obtain good photomicrographs it is essential that the dust particles be in one plane, free from Brownian movement and well dispersed. Since industrial dusts are seldom of a uniform size it is difficult to fulfil the first requirement. Comparisons have been made between the results obtained with the direct filar measurements and the photographic method on typical industrial dust samples. This comparison demonstrated that the simpler and less expensive filar method yielded practically the same results. Since the filar method fulfils the requirements of this problem it is suggested for use in such studies, unless permanent records are wanted, where photographs might well meet the requirement.

Composition of Industrial Dusts—The methods used in determining the composition of certain poisonous dusts, such as lead compounds, are amply discussed in the numerous publications dealing with these problems. Concerning the so-called fibrosis producing dusts, the work of the past 20 years on the problem of dust inhalation has demonstrated that, in general, the degree of health hazard associated with the inhalation of any dust, all other factors remaining constant, is dependent upon the mineralogical composition of the dust. For example, it is now established that the inhalation of certain types of dust, such as granite dust [29] may, in time, produce fibrosis of the lungs, frequently associated with tuberculosis. In other cases exposure to dust may result in the production of a far lesser degree of fibrosis without subsequent tuberculosis; this is true of cement dust.[30] And finally, there are certain types of dusts which produce little lung fibrosis, as typified by marble dust.[31] In general it has been found that those dusts which are high in quartz content are the ones which most readily produce a disabling fibrosis of the lungs.

The question of ultimate importance of silicates or combined silica (silica, sericite, talc, etc.) in the production of pathology is at present undergoing extensive study, but it is trusted that the methods of sampling and counting, enumeration, and sizing of dust particles above described will be found to be adequate for all injurious forms of mineral and other industrial dusts. Should it be ultimately shown that dusts less than 0.5 μ in diameter are a serious factor in the production of disease, resort may have to be made to the more refined methods of estimation referred to, and possible new methods devised.

Analytical methods for determining the chemical composition of some inorganic atmospheric dusts are referred to by W. A. Cook in another section of this report. It is to be noted, however, that a chemical analysis of a mineral dust will not disclose the various percentages of minerals existing in the dust. For example, a chemical analysis of granite dust will not reveal the percentage of quartz present in the dust, but will only tell the analyst the total silica, combined and uncombined. Hence, the close relationship

of the chemist, mineralogist, and petrographer to the situation must be kept constantly in mind. It is not considered, however, within the scope of this report to go into this feature.

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Advisability of Routine Laboratory Examination of Food Handlers

YOUR Committee on the Advisability of Routine Laboratory Examination of Food Handlers made a progress report in 1934 (see *Year Book 1934–1935,* page 113) on two aspects of the food handler problem, *i.e.*, an evaluation of the recommendations of health executives and the significance of food handlers as a source of infection. The recommendations of the Surgeon General's Advisory Board on Milk Ordinance were cited. These recommendations are part of the Milk Ordinance and Code of the U. S. Public Health Service. The committee appreciates that these regulations are based upon an extensive study of the milk handler problem. Mr. Abel of the Advisory Board made a careful survey of the food handlers' problem in Alabama. In his recommendations solicited by the committee in 1933, he made a valuable suggestion which the chairman had hoped to follow up; namely, a study of the use of dyes for determining the authenticity of specimens of feces in the search for typhoid carriers. There has been no opportunity to make this investigation.

Special investigations have been made by members of the committee: Mr. Mickel, *et al.*, on the detection of carriers among food handlers (*A.J.P.H.* 24:493, 1934) and laboratory examinations of milk handlers (*A.J.P.H.* 25: 557, 1935); Dr. Gilbert, on the practical limitations of examinations of specimens collected without regard to clinical history or epidemiological evidence (*A.J.P.H.* 24:193, 1934); Dr. MacNabb, on control measures in the municipalities of the Province of Ontario and routine examinations of food handlers in selected groups.

During the year a study of the incidence of *Beta hemolytic streptococci* from throat cultures of milk handlers was made by the Connecticut Department of Health and Yale University School of Medicine. The results of these studies will be reported in the *American Journal of Public Health.*

Routine health examinations—Although not strictly within the province of this committee, consideration of the control measures recommended by the health executives has been continued during the year. The New York City Department of Health rescinded the section of the Sanitary Code which provides for the yearly health examinations of food handlers. The control measure had been in effect for about twenty years. "The plan," quoting from Dr. Rice's report, "was adopted in 1915 in order to impress upon food handlers the rôle played by infection in the spread of communicable diseases and to encourage the practice of periodical health examinations." It is a significant fact that routine examinations of food handlers continue to be inaugurated by health executives for the same or similar reasons. Such examinations may include routine laboratory examinations of all applicants or only laboratory examinations when indicated by the medical examiner.

Significance of food handlers as sources of infection—In order to study further the significance of food han-

dlers as sources of infections, a questionnaire was sent to the state epidemiologists requesting information regarding the number, the infection, the source and mode of food-borne epidemics during 1930–1934 inclusive, and the number of cases and deaths. The committee wishes to thank the epidemiologists for their splendid response. In a number of instances, it meant special effort to accumulate the information.

In a surprising number of states, the records were incomplete or information unavailable. From an analysis by the committee of the replies, the following conclusions were drawn:

1. That many of the state departments of health are inadequately organized, not only for detecting outbreaks of food-borne infections, but also for placing them in their proper categories.
2. That the most important single food which may become a disease vector is raw milk.
3. That wherever a state department of health is well organized and proper search is made, a considerable number of outbreaks are found in which food is the vehicle of transmission.
4. That the number of these outbreaks, even in the imperfect manner in which they have been reported, is sufficient evidence to make the inspection of food handlers by some method or other seem worth while.

EVALUATION OF LABORATORY EXAMINATIONS

Laboratory examinations and tests are obviously for the detection of carriers and mild cases or for the confirmation of clinical findings. Laboratory examination as an aid to diagnosis needs no discussion. The need for laboratory examinations supplementing epidemiological investigations of food-borne outbreaks is recognized as essential. The value of routine laboratory examinations has been questioned, however.

The discussion of routine laboratory examinations for detection of carriers follows:

The index to the typhoid carrier is the typhoid case and a careful follow-up of convalescents and contacts would concentrate effort at the source of danger and eliminate the necessity of examining all food handlers for a possible carrier. In illustration of this, the experience of New York City is significant. Since 1915, a total of 565 typhoid carriers was discovered in New York City. Of this number, 308 were discovered as the result of epidemiological investigations made in connection with cases of typhoid fever, and 227 were discovered through stool examinations of patients after convalescence. In contrast to this, out of three and one-half million routine annual examinations made of food handlers during the past 18 years, only 30 typhoid carriers were discovered. This is less than one out of each 100,000 food handlers examined.

The specimens most frequently submitted to the laboratory from food handlers are for the control of enteric infections. These may be either blood serum for the Widal reaction or feces for *B. typhosus*, *B. enteritidis*, etc. The Widal has a limited value for it has been shown that carriers occasionally have no demonstrable agglutinins in the blood stream. The value of the examination of feces depends upon the number of specimens submitted and authenticity of the specimen. Bile is the most satisfactory specimen for the identification of a typhoid carrier. Laboratory examinations of routine specimens from food handlers are not recommended, except from raw milk handlers. However, routine examinations of raw milk handlers at regular intervals without other control measures do not render the supply of raw milk safe.

Septic sore throat and scarlet fever—Milk-borne outbreaks of septic sore throat and scarlet fever are of great

importance, but routine throat swab examinations for the detection of carriers are not practical. The wide distribution of hemolytic streptococci, and the multiplicity of strains makes classification necessary. The classification of hemolytic streptococci is a difficult procedure, involving time and special technic. Hence, the examination of throat swabs for identification of hemolytic streptococci is not recommended except under epidemiological direction.

Diphtheria—Diphtheria bacilli are of little significance in food-borne outbreaks. The detection of carriers has no practical value.

Tuberculosis—Food-borne tuberculosis is confined almost entirely to the bovine type as far as we know. Laboratory tests are not as efficient for diagnosing missed cases as physical examinations in conjunction with the skin test and X-ray.

Amebic dysentery—Carriers of *Entameba histolytica* still present some unsolved problems. How many persons contract the disease from another is not clear. The laboratory examination must be made by an experienced bacteriologist and on carefully collected specimens. The detection of *Entameba histolytica* in chronic amebiasis (the carrier) usually involves the examinations of scrapings obtained at sigmoidoscopy. Hence, laboratory examinations of specimens from food handlers are not recommended except under epidemiological direction.

It is felt by the committee that the indiscriminate collection and examination of specimens from food handlers are undesirable, and that such examinations should be made only where called for by epidemiological or clinical findings.

MINNA CROOKS YOUNG, *Chairman*
A. L. MACNABB
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Aspects of Veterinary Public Health

VETERINARIANS who become peculiarly involved with the work of public health usually will be found active in: (1) Meat inspection, (2) Milk inspection, (3) Bovine tuberculosis control, (4) Biologic production, (5) Immunology, (6) Bacteriology, (7) Serology, (8) Rabies control, and (9) Academic.

The veterinarian in general practice only contacts such specializations, casually. Tuberculosis, rabies, and brucelliasis constitute material of public health significance most frequently published in American veterinary journals. In such articles, the rule is that the public health aspects are incidental to more pertinent veterinary problems.

Under these circumstances, the veterinarian in public health has specialized away from his fundamental training for general practice and has become absorbed in logical groups or sections which include: (a) Laboratory Section, (b) Food and Nutrition Section, (c) Public Health Education Section, and (d) Instances of veterinarians who are health officers.

The loss of professional identity is not different from the case of the doctor of human medicine who has entered sections specializing in branches of public health. We can conceive that the creation of a separate section for veterinarians would only result in confusion, since the actual functions within the profession are so varied in actual health department practice.

An expression of opinion from the majority of veterinarians in public health work has so far been impossible. This is due to the fact that there is not available a registry of such men. We believe this should be one of the first endeavors of a committee during the coming year. Such a listing, with notations of the type of work engaged in, would alter the present obscure viewpoint to one of a more definite perspective.

Letters have been received from Missouri, Pennsylvania, New York, Illinois, Iowa, and Canada. Trends of such letters include such statements as:

1. "It does not occur to me that the Sections of the American Public Health Association should be set up with a view of giving any particular profession recognition."
2. "A section on veterinary public health would probably not be a flourishing one at least for some years."
3. "'Veterinary' is objectionable from the standpoint of linking such an activity directly with the practising veterinarian. We do not have physicians' public health sections."
4. "I do not consider it advisable to form a separate veterinary public health section immediately."
5. In Canada: "I do not think that there is any desire with us to form a separate section. . . ."
6. ". . . a section for veterinarians would scarcely seem in accord with the present policies any more than would a section for physicians."
7. ". . . it often happens that a veterinarian is the one most competent to discuss human infections of animal origin. Papers of this kind would be of general medical and bacteriological interest, and it does not seem to me they should be shut off by themselves in any way from the (Laboratory) Section."

Those favoring a separate section were few in number. Arguments favoring such a break from the Laboratory Section, etc., fundamentally reflect the feeling that the veterinary profession

does not obtain the recognition it should in the good work done. The following excerpt from a letter of December 14, 1934, was the result of careful checking with associates before the chairman expressed his general conclusion:

> "Those who teach Veterinary Hygiene in veterinary schools are very anxious to make public health officials aware of the fitness of the veterinary graduate to supervise milk and meat production. Therefore, I am voicing the desire of that body when I support those who believe that a separate section of the American Public Health Association for the consideration of the problems of food inspection should be formed."

Again, we can give voice to a strictly personal opinion as chairman of this committee. Why not inaugurate in the pages of the *Journal* a roster of veterinarians who are members of the American Public Health Association? Why not list the Fellows and the Associates? Many familiar names would be recognized in such a listing—names of workers who produced as "sanitarians" work of moment and importance to our general efforts in public service. An abstract section for articles pertinent to public health produced by veterinarians would remove some of the obscurity to the profession without in any way divorcing us from our chosen Sections.

The majority should formulate an opinion. For that reason this committee cannot offer more than a progress report. Once a roster of veterinarians is obtained, then can they be generally circularized, and a survey by questionnaire should result in a decision, during the year, as to the desire or lack of a desire for a separate section.

R. V. STONE, *Chairman*
A. L. MACNABB
I. A. MERCHANT

Resistance of Micrococci to Pasteurization*

ONE of the most commonly encountered groups of organisms in milk and other dairy products is the group of micrococci. The public health significance of this group, its effect on the chemical changes in the milk, and its relation to the sanitary production of milk, has been discussed by a number of workers. The results of these discussions based upon research have not been concordant in their conclusions. One of the difficulties of obtaining more concrete data with respect to the micrococci is due largely to the variability of individual strains with respect to their reaction to external influences, particularly heat. It must not be overlooked that other variable factors as age of culture, reaction of media, etc., may affect the heat resistance of this group.

The public health significance of the micrococci still remains a problem due to the variability with which toxins are produced by certain species of this particular genus. It has been found that the orange types (*Micrococcus aureus*) are more given to the production of toxins than any other of the species. However, there appears to be a variation also in this respect due probably to the type of medium and the source of nitrogen provided these organisms. For this reason, it is difficult to determine definitely the types which are of public health significance from this particular standpoint.

The ability of the micrococci to resist heat, particularly the pasteurization temperature, *viz.*, 145° F. for 30 minutes, is also a variable character. A study of the plates made from pasteurized milk in routine control work will generally reveal the presence of a certain number of micrococci. If the plates are allowed to stand at room temperature for several days, the micrococcus colonies frequently become more readily recognizable because of the development of lemon yellow or orange pigments. None of the species of this genus have been found to be thermophilic, *i.e.*, they will not grow at 55° C. (131° F.).

It is of interest to note that although these organisms are very common in milk and dairy products, only meager data are available as to their ability to resist 145° for 30 minutes.

Sternberg (1887) and Van Geuns (1889) were among the first to study definitely the heat resistance of the micrococci. The latter found that the yellow types in some cases were more resistant than the strains which failed to produce pigment. He also noted that certain strains were completely killed at 140° F. for 30 minutes, while other cultures resisted 145° F. when held for the same length of time. Dyar (1895) found a species of micrococcus which resisted 212° F.

From the standpoint of pasteurization, the work of Russell and Hastings (1902), as well as that of Orla-Jensen (1919), indicates that there are definite species of micrococci which will resist ordinary pasteurization temperatures. Russell and Hastings found a lemon yellow micrococcus which withstood pasteurization and required 169° F. to be killed. Orla-Jensen reports that

* Report of the Committee on Milk Pasteurization Studies of the Laboratory Section.

certain of the white liquefying types will resist 145° to 165° F. for 15 minutes.

More recent work has shown that the yellow type of micrococci are more resistant to pasteurization temperatures than any of the other species. Robertson (1927), Hammer and Trout (1928), Hucker (1928), Eglinton and Yale (1931), and more recently Myers,* have found that the yellow or orange types are resistant to a temperature of 145° F. for 30 minutes.

The members of this genus are more heat resistant than any other of the generally encountered non-spore-bearing organisms. Since many species of this genus have thermal death times considerably greater than 30 minutes at 143.5° F., they are not killed by efficient pasteurization. Dotterrer (1923) was one of the first to point out that where producers bring in milk with a high bacterial count, the counts from the pasteurized milk may also be high because large numbers of bacteria, usually micrococci, may be so heat resistant that they are not killed by the pasteurization. Dotterrer, Myers, and Phelan * have made extensive use of routine laboratory tests to locate producers whose milk is contaminated with heat resistant organisms. It is possible also by carefully controlled laboratory

* Private communications.

TABLE I

THE THERMAL DEATH POINTS OF MICROCOCCI

Date	Organisms	Medium Used	Temperature ° F.	Length of Holding Time, Min.	Per Cent Killing	Remarks	References
1887	*Micrococcus sp.*	Broth	145	10	0	Used special bulbs	Sternberg, 1887
	Micrococcus sp.	Broth	140	**30**	**0**	" " "	
1889	*Micrococcus sp.*	Broth	145	**30**	**100**	Used test tubes	Van Geuns, 1889
	Micrococcus sp.	Broth	145	30	95	" " "	
	Micrococcus from water	Broth	145	30	100	" " "	
	White *Micrococcus sp.*	Broth	145	30	100	" " "	
	Staphylococcus pyogenes	Broth	145	30	100	" " "	
	Staphylococcus pyogenes albus	Broth	145	30	90	" " "	
	Staphylococcus pyogenes aureus	Broth	150	30	100	" " "	
	Staphylococcus pyogenes citrens	Broth	145	30	100	" " "	
1895	*Micrococcus sp.*	Broth	212	—	?	Used test tubes	Dyar, 1895
1902	Lemon yellow *Micrococcus sp.*	Milk	169	—	100	Used test tubes	Russell and Hastings, 1902
1919	*Tetracoccus casei*	Broth	150	15	0	Used test tubes	Orla-Jensen, 1919
	Tetracoccus casei	Broth	165	**15**	**80**	" " "	
	Tetracoccus casei	Broth	165	15	15	" " "	
	Tetracoccus liquefaciens	Broth	145	15	0	" " "	
	Tetracoccus liquefaciens	Broth	145	15	0	" " "	
1927	*Micrococcus conglomeratus*	Milk	151–4	30	100	Used special bulbs	Robertson, 1927
1928	*Micrococcus epidermidis*	Milk	145	30		Used test tubes	Hucker, 1928
	Micrococcus varians	Milk	158	30	0	" " "	Hammer and Trout, 1928
	Micrococcus varians	Milk	176	30	100	" " "	
	Micrococcus luteus	Milk	158	30	0	" " "	
	Micrococcus luteus	Milk	176	30	100	" " "	
	Micrococcus candidus	Milk	145	30	0	" " "	
	Micrococcus luteus	Milk	145	30	0	" " "	
1931	Yellow *Micrococcus sp.*	Milk	143	30	10	" " "	Eglinton and Yale, 1931
1935	Yellow *Micrococcus sp.*	Milk	145	30	10	" " "	Myers, 1935
	Staphylococcus aureus	Milk	145	30	98	" " "	

pasteurization in connection with commercial plant pasteurization to determine whether high colony counts on the pasteurized milk are due to the presence of large numbers of heat resistant bacteria or to defective or inadequate commercial pasteurization.

A study of the micrococci resisting pasteurization may throw considerable light on the conditions under which the milk was produced and pasteurized. In most instances the micrococci are inhabitants in the normal udder and, according to Breed (1928), the normal micrococcus flora of the udder may be several thousands per c.c. without any evidence of an infection being present. Among these udder types are to be found the yellow or the most resistant varieties of micrococci. For this reason, when commercial pasteurization is carried out effectively as demonstrated by comparison of pasteurization in the laboratory of small samples with samples secured from the plant pasteurizer, and heat resistant micrococci found still to be present, the source of these organisms may be the bovine udder.

However, Wickham and Widmer (1931) feel that thermoduric organisms are derived from improper sterilization of the utensils and that their presence should be considered when quality is used as a basis for payment to producers. They feel that shippers who deliver milk at the pasteurizing plant free from thermoduric organisms should be given preference over shippers furnishing milk that contains large numbers of heat resistant bacteria.

When micrococci are present in pasteurized milk they may be of the heat resistant types which will resist 145° F. for 30 minutes (Table I), or they may be of the border-line heat resistant varieties which survive pasteurization if this process is not carried out efficiently.

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ROBERT S. BREED, *Chairman*
G. J. HUCKER, *Secretary*
L. H. BURGWALD
MAC H. MCCRADY
R. P. MYERS
R. C. THOMAS

Coördinating Committee on Standard Methods

THE membership of the Coördinating Committee remains exactly as it was constituted at the Indianapolis meeting. The selections made then were so fortunate that no change has been contemplated. Each of the Chairmen of the three Standard Methods committees has put into his work that combination of sincerity, devotion, and enthusiasm which is so rare and precious an asset to any organization. The keen interest of the Section Secretary in every plan and development has been a constant source of constructive suggestion. As their respective reports will show, our committees are making the progress the Association demands of us.

The Water committee, under the Chairmanship of Dr. John F. Norton, has, during the year, completed an important coöperative investigation of various types of selective culture media that had been suggested for use in the bacteriological tests. In addition certain chemical factors have been carefully studied. The results and recommendations will be presented in detail at this meeting.

The Committee on Dairy and Food Products, under the Chairmanship of Dr. Robert Breed, has been engaged in fundamental studies based chiefly upon the composition of the culture medium and the temperature of incubation used in the bacteriological examination of milk. The results of their work are so convincing that there is no doubt that the new methods elaborated should be adopted. This will involve changes in significance of numerical results and their interpretation and may even require amendment of milk codes and regulations. Even with the fundamental support of accurate laboratory demonstration such innovations will not gain general acceptance by mandate alone. Their adoption into current laboratory practice will result only from a persistent and carefully planned campaign of education, continued until those most concerned themselves demand the change.

It is fortunate that we have, in the chairman of our milk committee, a man competent, not only to recognize and develop more accurate laboratory analytical procedures but one also with the experience and judgment which enable him to organize the coöperative publicity required to bring about, in the most effective manner, general utilization of the new methods developed by his committee.

Accomplishments of the committee during the year will be described in detail by Dr. Breed and his referees. Their reports of fundamental studies will not, however, concern the milk program alone. Equally interesting and important progress has been made in the work upon the "Sanitary Oyster."

The reports already published by referees of Dr. W. D. Stovall's Committee on Diagnostic Procedures and Reagents illustrate the type of work that is being done by that group. The field being covered by this committee comprises all the routine technical procedures of the public health laboratory

(except, of course, those concerned with water and milk). The progress made during the year will be demonstrated by the contributions to be read at this meeting. It is predicted confidently that when Dr. Stovall has collected the approved reports of his referees he will have produced a laboratory manual equal in every way to the best publications of our Association.

As a result of the continuing policy of constant investigation and revision by referees who are outstanding in their special fields, our Standard Methods Committee reports maintain a unique position in laboratory literature.

In the chairman's report for last year it seemed necessary to refer to certain misunderstandings concerning the scope of our standard methods work. This year it seems unnecessary to refer to such discussions but merely to observe that there have been none.

At a meeting in Chicago, last December, Dr. Stovall asked several of the referees of his committee who were present to meet him at an informal conference. The discussion of work in which there was common interest proved to be most stimulating. As a result of that experience it has been decided to bring all Laboratory Section Standard Methods Committee members together at a dinner meeting this year. The purpose, of course, is chiefly that of making those who are creating public health laboratory history well acquainted with one another. It is not unlikely that this dinner conference will become one of the established features of subsequent Annual Meetings of the Association.

The only recommendation I have to make concerns the appointment of a new committee. Studies are being reported with greater frequency concerning the variable reactions of laboratory animals to experimental infection and to antigenic substances. There is already in the literature abundant information concerning the relative resistance of the species to infection and concerning differences in strains or breeds of the same animal species. Often we find it advisable to breed our own animals in order to have a pure line for special work. The ages and weights of animals most suited to the particular purpose are often exceedingly important. There are matters concerning the general care of laboratory animals which are not common knowledge. The same may be said concerning normal blood counts, anatomy, and physiology, as well as autopsy methods. Such information may be difficult to find at the time it is most needed and may require considerable literary research. We need to have it collected into a single volume by a group of referees best qualified and working together as our other Standard Methods Committees have been doing. It is recommended, therefore, to the council of the Laboratory Section that, in accordance with the rules and regulations governing Standard Methods Committees, a new committee be formed to be known as the "Standard Methods Committee on the Biology of Laboratory Animals," of the Laboratory Section.

As Chairman of the Coördinating Committee, I can express nothing but the deepest gratitude to all those with whom I have been in correspondence during the past year. They have made my labors very light—I have been on the receiving end throughout—and they have created a most optimistic belief in a productive future for our Laboratory Section.

A. PARKER HITCHENS, *Chairman*
ROBERT S. BREED
JOHN F. NORTON
W. D. STOVALL
FRIEND LEE MICKLE (*ex officio*)

Examination of Dairy and Food Products*

Present Status of the Report on Standard Methods for the Examination of Dairy Products—One of the most important activities of your committee during the past year has been the maintenance of contacts with other committees, sections, and associations interested in the development of laboratory methods for the examination of dairy products. There are so many of these that only the most important can be mentioned. Contact has been maintained with the work of the Association of Official Agricultural Chemists in the field of chemical analysis of dairy products and they have been urged to broaden the scope of their work to include methods for the detection of nutritionally important mineral elements in dairy products. Arrangements to include methods for the bioassay of milk have been made through the Food and Nutrition Section of this Association, and a report on these methods is to be given during the symposium on Standard Methods for the Examination of Dairy Products.

Through coöperation with the Committee on Bacteriological Methods of the International Association of Dairy and Milk Inspectors, a questionnaire has been distributed on the use now being made of the tentative Standard Methods for the Examination of Ice Cream. A report on this matter is to be made before the latter association at its annual meeting which follows immediately after the meeting of the American Public Health Association. At the same time, the chairman of your committee is to make a report on the efforts that are being made to bring about an international standardization of laboratory methods in the dairy field. International activities have led to the organization of a symposium on this subject which your chairman has been asked to open before the Second International Congress for Microbiology that is to be held in London in July, 1936.

A report on the work of the Standard Methods Committee for the Examination of Dairy and Food Products has also been given during the year before the Laboratory Section of the International Association of Milk Dealers, and before the American Dairy Science Association. Contact has also been established with the Committee on Standard Methods for Milk Analysis of the Canadian Public Health Association, and Committees on Research of the Ice Cream Manufacturers' Association, and of the American Association of Creamery Butter Manufacturers.

The secretarial assistance provided by the American Public Health Association has permitted us to maintain an active address list of the nearly 1,000 laboratories in the United States and Canada that utilize standard laboratory procedures for the examination of dairy products. Four reports and the questionnaire referred to above have been distributed to these laboratories during the past year.

Because of the demand for the inclusion of methods of making bacterial

* Report of the Standard Methods Committee.

counts for ice cream and for the inclusion of methods for the detection and microscopic examination of sediment in heavy cream and butter, and for making counts of yeasts and molds in butter, it is planned to broaden the scope of the next report. It has been suggested that the next edition should be called Standard Methods for the Examination of Dairy Products rather than to use the older title of Standard Methods of Milk Analysis. An Associate Referee to cover ice cream methods was appointed some time ago and Dr. E. H. Parfitt has recently been appointed Associate Referee to cover methods for the examination of butter. Their activities in this field are to be limited, as are the activities of the committee, to the development of methods that are useful in official control work.

During the year, several investigations that have a bearing on standard laboratory methods for the examination of dairy products have been completed under the general supervision of your committee, or by independent workers. The most important of these studies that are known to your committee are—

1. A study of the usefulness of samples of milk taken for bacterial analyses from weigh vats at receiving stations. (To be discussed at the meeting of the Laboratory Section of International Association of Milk Dealers.)
2. Further studies on the composition of standard agar.
3. Further studies on the optimum temperature of incubation to use in case an improved agar is selected.
4. Studies on methods of improving the standardization of the methylene blue reduction technic.
5. Studies on the efficiency of the tentative methods outlined for detecting organisms of the colon group in dairy products. (Reports on studies mentioned under 2, 3, 4 and 5 are to be reported at the symposium on Standard Methods for the Examination of Dairy Products.)
6. Studies on the significance of the presence of organisms of the colon group in samples of raw milk that are not more than 4 hours old. (To be reported at the meeting of the International Association of Dairy and Milk Inspectors.)
7. Studies on the usefulness of the colon test as a means of detecting inefficient pasteurization. (To be reported at the New York State Dairy and Milk Inspectors' Association, and at the International Association of Milk Dealers.)

It is expected that the next edition of the *Standard Methods for the Examination of Dairy Products* will utilize the conclusions reached in these or subsequent investigations in the same field.

The reports by Dr. Hagan on methods of detecting tubercle bacilli in milk and by Dr. Huddleson on methods of detecting Brucella organisms in milk (*Year Book, 1934–1935*) have been favorably received and it is planned to include these with slight modifications in the next edition of the milk report.

Present Status of the Shellfish Report—The revision of the 1922 first edition of Standard Methods for the Examination of Shellfish, prepared by a Committee of which the late Professor Gorham was chairman, was approved by the Laboratory Section at Pasadena in September, and by the Committee on Research and Standards of the Association in December, 1934, for use as a basis for further revision. This second edition of the 1922 report has not been published, but has been distributed in mimeographed form with a questionnaire to all persons in the United States and Canada believed to be interested in a revision of methods. A conference of interested persons was held in Washington, D. C., at which a compilation was made of answers to a questionnaire sent out by Dr. C. A. Perry. A symposium on this general subject has been arranged for the Milwaukee meeting and this compilation will form an important part of the progress report to be given at that time.

General—The work of the committee has been greatly facilitated by the pro-

vision made by officers of the Association of funds which have permitted the holding of a conference on Dairy Products Methods at Geneva, N. Y., April 8, 1935, and a conference on the Shellfish Report held jointly with representatives of the Food and Nutrition and Public Health Engineering Sections at Washington, D. C., April 18, 1935. The first printing of 2,000 copies of the Sixth Edition of the Milk Report was sold out during the year and the report was reprinted in an edition of 3,000 copies. This edition is expected to last until 1937 at which time it is planned to present the manuscript of a new edition for approval by the Section and the Association.

ROBERT S. BREED, *Chairman*

Referee for:

Methods of Detecting Specific Types of Bacteria in Dairy Products, MAC H. MCCRADY

Methods of Counting Bacteria in Dairy Products, A. H. ROBERTSON

Chemical Methods of Examining Dairy Products, F. C. BLANCK

Shellfish Examinations, C. A. PERRY

Associate Referee for:

Methods of Identifying Streptococci in Dairy Products, G. J. HUCKER

Bacteriological Methods for the Examination of Ice Cream, F. W. FABIAN

Milk Sediment Test, CARYL C. CARSON

Methylene Blue Reduction Test, H. R. THORNTON

Methods of Examining Milk for Tubercle Bacilli, W. A. HAGAN

Methods of Examining Milk for Evidences of Brucella Infection, I. FORREST HUDDLESON

Microbiological Methods for the Examination of Butter, E. H. PARFITT

Examination of Shellfish for Fecal Pollution*

A Revised Bacteriological Procedure

THE present method for bacteriological examination of oysters for fecal pollution has been severely criticised by many of those who have given extensive and studious consideration to it. This method was the outgrowth of studies begun by a committee of the American Public Health Association and reported on in 1912 and 1916. The method was finally adopted by the Laboratory Section at San Francisco in September, 1920, and published in Volume 21 of the official JOURNAL of the Association in 1922. Minor changes, particularly in the phraseology of the method, were made by Professor Gorham at Washington in 1922, but these changes did not make the method any more suitable for its primary purpose of measuring fecal pollution and did not, consequently, satisfy the growing criticism.

A mimeographed revision of the 1922 standard procedure was distributed with a questionnaire on February 10, 1935. Seventy copies of the method and questionnaire were sent to all persons in the United States and Canada known to be interested. Fifty-three of these were sent to individuals in 40 different laboratories and to 17 other persons thought to be interested in an official method for the examination of shellfish. Twenty-five questionnaires were returned with answers to some or all of the questions while 10 persons sent replies by letter who did not consider themselves qualified to answer the questions. It may be assumed, therefore, that the replies to this questionnaire represent the attitude of those who have had most experience with the present standard method. The replies to the questionnaire were compiled in as brief a manner as possible as a basis for further criticism.

A conference of some of those actively interested in a new standard procedure for shellfish was held at the Army Medical School in Washington, D. C., on April 18, 1935. A very free discussion of the problem of new standards took place. Summaries of the answers to the questionnaire were used as a basis for criticism and discussion. A copy of the report on this conference and of the questionnaire, together with the summary of the replies to the questionnaire, was immediately sent to interested persons for their further criticism.

APPROACH TO NEW PROCEDURE

The guiding policy in formulating a new procedure has been first to determine the attitude of those who have had extensive experience and particularly those who have given studious thought to the matter, secondly to review these attitudes critically and finally, with this background, to attempt to evolve a procedure.

* Progress Report of the Referee on Shellfish Standards for the Committee on Standard Methods for the Examination of Dairy and Food Products.

PRIMARY PURPOSE OF A PROCEDURE

It is obvious to all that the primary purpose of a standard procedure is to provide a method whereby the best possible knowledge may be had of the amount of fecal pollution in shellfish. The chief danger is typhoid fever. Lesser hazards are other enteric infections such as bacillary dysentery, paratyphoid fever, and cholera. It is also thought by some that there is a danger in transmitting other infectious diseases such as tuberculosis, syphilis, and skin infections, if people with these infections open shellfish. Physical and laboratory examinations for these diseases are, however, made routinely on employees in shellfish establishments, and it is not necessary to provide any special procedure for this purpose. The purpose, therefore, of this report, is to determine a procedure whereby the freedom of oysters and other shellfish from possible enteric organisms may be estimated.

It has not been considered necessary to publish the full report of the author in which the evidence both in favor of these and other minor changes, and that opposed to them was given. Since the part dealing with the proposed use of *Escherichia coli* rather than the colon group represents a more fundamental change than any other, and since the reasons for this change are of interest to many besides those interested in shellfish standards, this and a few other parts of the paper believed to be of general interest are given below.

The fact that the colon group has been found to be of so little value in estimating fecal pollution in oysters has made it mandatory that a more satisfactory index be found. The fact that *Escherichia coli* is the one member of the colon group which is generally accepted as an unquestionable and typical fecal type, and the fact that this organism has not been found to any extent in oysters, even those giving exceptionally high scores, unless such oysters were known to come from polluted areas, has naturally led many to believe it would be more satisfactory for this purpose. An impasse has been reached where it would seem that either some more suitable indicator of significant fecal pollution must be found, or the bacteriological examination of oysters must remain in a position of very questionable value, and be abandoned or discredited by many able workers. The use of scores based on the colon group as an indicator of pollution in oysters directly from their beds is already obsolete in Maryland and Canada. In this connection it is of interest and value to know that *Escherichia coli* was adopted by an international conference on a standard procedure for the examination of edible mollusks at Middleburg, Holland,[1] in September, 1932. Typical *Escherichia coli* is described as (1) a Gram-negative, nonsporing bacillus, (2) producing acid and gas from lactose, and (3) glucose, (4) producing indol, (5) giving a positive methyl red, (6) a negative Voges-Proskauer test, and (7) failing to grow in Koser's citrate medium. These are precisely the characters recommended by the writer[2] in 1928.

Realization of this situation has been the impetus which has resulted in a study for a practical method for the determination of *Escherichia coli* in Maryland. The fact that oysters, taken from sources of unquestionable purity as judged by sanitary surveys and bacteriological examination of the oyster waters, would frequently have excessively high scores during the warm summer and fall months together with the fact that the bacteria responsible for such scores were found to be almost exclusively *Aerobacter cloacae, Citrobacter sp., Aerobacter aerogenes*-like types and various intermediate colon group types to the exclusion of the

universally accepted fecal type, *Escherichia coli*[2, 3] indicates that the scores of oysters would be more significant if based on *Escherichia coli*. Not only is this true for shell oysters taken directly from their beds but it is a well established fact that in many instances the same type of colon flora is found in shucked oysters.

Since there is general agreement among bacteriologists that *Aerobacter cloacae* is one member of the colon group which is seldom found in human stools and is, therefore, generally accepted as not being a fecal type, and since this organism so often composes a large part of the colon group responsible for the type of high scores referred to,[2] there can be little objection to referring to the non-*Escherichia coli* members of the colon group in oysters as non-fecal types. It is obvious, therefore, that such non-fecal types find a natural habitat in oysters where a suitable food environment exists. Such forms are frequently the cause of excessively high scores in shucked oysters and undoubtedly are the cause for numerous complaints, investigations, and rejections where, as a matter of fact, they are probably without significance as indicating fecal pollution. They are found in large numbers in the dirt from unsanitary utensils, and easily increase in oysters if kept at comparatively warm temperatures. They may be present in large numbers to start with, and being able to tolerate a higher acidity than *Escherichia coli* they are, apparently, more characteristic of spoilage. This is the situation that has convinced many that *Escherichia coli* would be a more satisfactory index of fecal pollution in oysters.

In this connection it is of interest to review the attitude of those answering this question in the questionnaire and to know the attitude as expressed at the Washington conference. Of 24 persons answering the question, 14, or 57.3 per cent, favored the use of *Escherichia coli*, while 2 others, making 66.7 per cent, favored its use along with the colon group. Five, 21 per cent, favored the use of the colon group, while 3 others, a total of 33 per cent, considered the use of *Escherichia coli* impractical.

A very free discussion of this question took place at the conference and great interest in the problem was evident. In view of the acknowledged very limited value of the scores of oysters as now based on the colon group and the conviction that many members of this group are without significance from the standpoint of fecal pollution, the general opinion of the conference was in favor of using *Escherichia coli*, provided a satisfactory technical procedure could be developed.

In realization of the fact that scores based on the colon group are of no practical value, routine examinations for *Escherichia coli* (so-called fecal scores) rather than the colon group were instituted in Maryland in May, 1934. A great deal of work has been done since 1928 in the development of the Eijkman test[2] in the Maryland bacteriological laboratories because this seemed to offer the most practical approach to the problem of isolating *Escherichia coli*. A new Eijkman medium was developed and reported in 1933.[4] A report on the practical application of this test in the examination of oysters, crabmeat, and other substances was made in the JOURNAL of this Association in June, 1935.[5] A further report of the application of the test to the isolation of *Escherichia coli* from sewage appeared in the *Journal of Bacteriology*.[6]

Since the use of *Escherichia coli* seemed to be the key to the development of a new shellfish procedure, and since considerable work had been done in Maryland for some months, the services of Dr. Milward Bayliss were

secured for 2 months from funds provided by the Committee on Research and Standards to help compile the Maryland data. It was planned to include other data but these could not be secured.

The Maryland data were compiled to demonstrate what correlation, if any, there might be between pollution as estimated from sanitary survey information and pollution as indicated by *Escherichia coli* and by the colon group. A short report of these observations with charts has been submitted for publication in the *Journal* of the Association. Data were compiled for 4 rivers in Maryland, and a single table for all *Escherichia coli* data available has been prepared. In brief, these data indicate a close correlation between the estimated degree of fecal pollution as judged by sanitary surveys and as indicated by *Escherichia coli* densities in oysters, while there is little or no such correlation on the basis of the colon group. For the oyster producing waters there is a much closer correlation between observed pollution and the colon group, but even here there appears to be a much better one on the basis of *Escherichia coli*.

The Maryland data are too limited to warrant dogmatic conclusions but they justify further study.

STANDARD PROCEDURE FOR EXAMINING SHELLFISH WATERS AS WELL AS SHELLFISH

Practically all of those replying to the question, "Should a standard procedure for shellfish include one for shellfish waters?" indicated that a procedure for examination of the waters should be a part of standard methods for the examination of shellfish. Two only, felt that since the Association already has standards for the examination of water this should not be included. The principal reasons why a separate procedure is needed may be listed as follows:

1. The present standard method has been developed for drinking water; it has not been found satisfactory for estimating the quality of water suitable for growing shellfish.

2. Most of those concerned with the shellfish problem believe that *Escherichia coli* rather than the colon group would be a more suitable index of the type of pollution with which they are concerned.

3. There is general agreement that the same bacterial index of pollution should be used both for shellfish and shellfish waters.

The sentiment of the conference was in accord with the above summary of the attitude of those answering the questionnaire. The Maryland data on the relative value of *Escherichia coli* and the colon group support these points.

RECOMMENDED LIMITS FOR POLLUTION

The opinions of those with experience in shellfish control work differ sharply on the question of whether limits should be defined for pollution.

About two-thirds (15) of those ananswering this question favored the adoption of bacterial limits. A number indicated that such bacterial limits, however, should be based on *Escherichia coli*. Six were opposed to the established bacterial limits.

The opinion voiced at the conference was in opposition to stringent definition of standards. It approved, however, the inclusion of certain carefully phrased recommendations made on limits for pollution arrived at by those responsible for official control work.

In so far as procedure and limits of pollution are so intimately a part of each other, in so far as those who are best qualified to arrive at a desirable standard procedure are probably also best qualified to judge the significance of the practical application of this pro-

cedure, and in so far as a number of those concerned with the problem of evaluating bacterial results and determining pollution desire advice in this matter, the adoption of some sort of guide would seem to be useful. Such a recommendation should, however, follow the application of the new procedure in a relatively large series of examinations made on shellfish and shellfish waters of various degrees of pollution.

USE OF WHOLE OYSTER RATHER THAN SHELL LIQUOR

The question of whether an examination of shell liquor gives a proper idea of pollution in oysters and other mollusks has received considerable thought in this country, in England, and elsewhere. In Japan,[7] the American procedure has been used and will be adopted with certain modifications, chiefly the use of the whole oyster rather than shell liquor. In Germany,[8] there is no standard procedure and examinations by one of two private companies are confined to direct microscopic examinations of stained tissue for spoilage. In England, there are likewise no standard procedures comparable to those of the United States. The Bacteriological Standards of the Worshipful Company of Fishmongers [9] are, however, almost tantamount to such standards. Their procedures have been reviewed and approved by representatives from the Royal Society, and the Oyster Merchants' and Planters' Association, as well as from the Ministries of Health and of Agriculture and Fisheries in 1924. The procedures represent the long experience and thought of Sir Alexander Houston, Dr. Klein, Professor Eyre of the Fishmongers Association, Sir Frederick Andrewes of the Royal Society, and Professor R. Tanner Hewlett, representative of the Oyster Merchants' and Planters' Association. A most important point in the procedure is the use of the whole oyster. This procedure has, however, recently been superseded in reality by the recommendations of the Middleburg conference.

It is pertinent that under the auspices of the Conseil International pour Exploration de la Mer [1] at a conference held at Middleburg, Holland, in September, 1932, a standard method for the bacteriological examination of edible mollusks was considered. This conference was represented by delegates from Belgium, England, France, Germany, Holland, Irish Free State, and Portugal who were for the most part experts in this type of work. The procedure adopted by this conference is excellent in most regards and should receive careful consideration in the formulation of our own revised methods. It is noteworthy in connection with the point under discussion that the procedure adopted calls for discarding the shell liquor and using only the minced bodies of the mollusks. While a question may be raised on the validity of discarding the shell liquor, all evidence indicates the soundness of using the minced body of the oyster.

In the United States, the relative merits of using the whole oyster rather than the shell liquor for examination have been investigated particularly by Dowd and Glancy [10] of the Pease Laboratories, by Tarbett [11] of the U. S. Public Health Service, and, more recently, by the author and a member of his staff.[12] None of the results of these studies have been published, but the writer has had an opportunity to review the work of Dowd and Glancy and is familiar with Tarbett's conclusion that the whole oyster should be used. The studies made in Maryland confirm Tarbett's conclusion. The answers to this question in the questionnaire were not very conclusive, an equal number favoring the use of the whole oyster and the continued use of the shell liquor. The opinion of the

conference was strongly in favor of the use of the whole oyster.

USE OF MOST PROBABLE NUMBER VS. THE SCORE FOR EXPRESSION OF RESULTS

The score method of expressing the number of colon group bacteria in oysters has been subject to considerable criticism. While there is now general familiarity with oyster scores among those concerned with the sanitary control of shellfish, and there might consequently be some objection to changing a system so well established, yet this argument is not entirely valid. In the first place, of 22 persons expressing their attitude on this point in the questionnaire, only 9, or 41 per cent, favored retention of the score system against 13, or 59 per cent, who favored the use of most probable numbers. Those attending the conference were also in favor of expressing results as most probable numbers. A point of practical consideration is the association of certain scores, particularly a score of 50, as indicative of significant pollution. While such associations have been discredited, yet the association will remain in many minds for a long time. The transition to a new basis for measuring pollution as *Escherichia coli* will of course necessitate new standards. It is believed that this transition can be effected much better if results are expressed in most probable numbers. The score system is already obsolete in several places, notably Canada and Maryland where results are expressed as most probable numbers, and the score system is used in Maryland only for interstate purposes. It is proposed, therefore, that results for both shellfish and shellfish waters be expressed in terms of most probable numbers.

SUMMARY

On the basis of a questionnaire, conference, and consideration of studies made both in the United States and certain foreign countries, the following principal changes in the present standard procedure for the examination of shellfish are proposed.

1. A new procedure should include at least, such edible mollusks as oysters, clams, and mussels.
2. *Escherichia coli* rather than the colon group should be the index of pollution for both shellfish and shellfish waters.
3. A new procedure should include methods for the examination of shellfish waters as well as shellfish.
4. The whole oyster rather than just the shell liquor should be examined.
5. *Escherichia coli* results should be expressed as most probable numbers rather than as a score.
6. Certain recommendations should be made in regard to amount of pollution which should ordinarily be tolerated.

A procedure incorporating these principles has been drawn up for trial during the ensuing year, during which time it is hoped its value for testing oysters, clams, and mussels may be determined and such changes made in it as will best adapt it to the requirements of practical application.

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C. A. PERRY, *Referee*

Procedures for the Detection of Brucella in Milk*

EXAMINATION OF MILK (COW, GOAT AND SHEEP) FOR BRUCELLA AGGLUTININS

Collection and preparation of milk samples—Milk samples are collected, preferably from individual quarters of the udder, in clean test tubes or vials. The strippings should never be used. The tubes should be filled to only one-half their volume. Let the samples stand from 6 to 8 hours to permit the cream to separate. Remove as much of the cream as possible with a pipette. Add rennet (1 per cent solution), allowing 2 drops for each 5 c.c. of milk, to each tube of milk, and mix thoroughly. The tubes may be placed either in an incubator at 37° C. or in warm water at the same temperature. If the tubes are placed in a slanting position, the curd will settle to one side, and the clear milk serum with which the test is to be made will separate in about 2 hours. In order to obtain a serum free from particles of casein, the samples should be placed in an icebox or cold place for from 6 to 8 hours before testing. Sour and decomposed milk should not be used, as the results are not reliable. Neither should milk be used in which the curd has become partly digested as this interferes with the test. Colostrum is unsatisfactory because of the difficulty of separating the serum and the possible occurrence of nonspecific agglutinins.

The Agglutination test—One may choose either of two methods, namely the test tube or rapid agglutination test.

Test tube method—A smooth strain of *Brucella abortus* is grown on liver agar slants for 48 to 72 hours at 37° C. The growth is removed and suspended in physiological salt solution containing 0.5 per cent phenol. The final turbidity of the suspension should measure 7 cm. by the Gates wire loop method. Place 2 c.c. of the antigen into a series of 5 agglutination tubes. To the first tube add 0.08 c.c. of milk serum, the second 0.04 c.c., the third 0.02 c.c., the fourth 0.01 c.c., and to the fifth 0.005 c.c. These amounts of milk serum added to 2 c.c. of antigen will give approximately, dilutions of 1–25, 1–50, 1–100, 1–200, and 1–400. No further dilutions are necessary in making routine examination of milk for *Brucella* agglutinins.

Serum and antigen are mixed thoroughly in the tubes and incubated for 48 hours in a 37° C. room.

The following system may be used in recording changes in the stability of the antigen: + = complete sedimentation; P = incomplete sedimentation; — = no sedimentation. The presence of infection in the udder is indicated by complete agglutination in a 1–50 dilution or above of serum.

Rapid agglutination test [1]—The rapid agglutination test offers a simple and accurate method of detecting *Brucella* agglutinins in milk from any species of animal infected with any one of the three species of Brucella.

Materials needed — (1) darkfield illumination box and glass plate ruled into squares; (2) 0.2 c.c. pipettes graduated in 0.01 c.c.; (3) clean toothpicks for mixing milk serum and anti-

* Supplementary report of the Associate Referee on Methods of Examination of Milk for Evidence of Brucella Infection, for the Committee on Standard Methods for the Examination of Dairy and Food Products.

gen; (4) properly standardized antigen and standardized dropper pipette.

Technic—In beginning the test, arrange the serum samples in a row parallel with the darkfield illumination box. The glass plate, with the etched squares upward, is placed over the opening of the box and the identification number of the serum sample marked with a wax pencil on either the top or bottom of the row of squares used.

With a clean 0.2 c.c. pipette, draw up serum from the first milk sample to the zero mark on the pipette. Beginning in the bottom left hand square of the plate, place the following amounts of serum in the succeeding squares toward the top: 1st square, 0.08 c.c.; 2nd square, 0.04 c.c.; 3rd square, 0.02 c.c.; 4th square, 0.01 c.c.; 5th square, 0.005 c.c.

This manner of placing the serum brings the smallest amount farthest from the heat of the electric bulb in the box, reducing the rapidity of drying of the smallest amounts of serum. The procedure is continued, using the next set of vertical squares and a separate pipette for each sample. The best results are obtained by testing only 4 or 5 samples at a time as otherwise the small amounts of serum dry out too much before the test is completed.

If the pipette has been placed deep in the serum, there will be some serum which will collect on the outside at the tip. For accuracy, this should be removed by touching the tip of the pipette against the lip of the vial.

After thoroughly shaking the bottle of antigen, remove a dropper full of the antigen. Holding the dropper in a vertical position, add one drop to each amount of serum on the plate. Care should be taken to hold the dropper in a vertical position since holding it at another angle will make a considerable difference in the amount of antigen delivered. Always replace the dropper directly in the vial of antigen.

Then with a clean toothpick mix the serum and antigen, using a new toothpick for each sample. Always start at the top of the plate in the square containing the smallest amount (0.005 c.c.) of serum and continue downward to the largest amount. Spread the mixture over about three-fourths the area of the square without coming in contact with the etched dividing lines.

Immediately after the samples have been mixed, remove the plate from the box and tilt slightly backward and forward slowly for about 5 minutes. Place the plate on the box, turn on the light, and record the results.

The reactions stand out very clearly. It is not difficult to distinguish between complete clumping of the antigen and different degrees of incomplete clumping. A negative serum causes no flocculation of the antigen. There are often encountered, however, sera which produce a trace of flocculation in the 0.08 c.c. amount.

Immediately after using, the pipettes should be rinsed several times with fresh water until thoroughly clean. Then boil in distilled water and drain all the water out before using again. The glass plate may be cleaned with cleaning powder and brush, after which it is rinsed with distilled water and dried. By having several clean plates available, one can proceed with the testing of additional samples without delay. Absolute cleanliness of glassware is essential.

Interpretation of the test—Rapid antigen has been standardized for use with undiluted serum in the following amounts: 0.08 c.c., 0.04 c.c., 0.02 c.c., 0.01 c.c., and 0.005 c.c.

In the test tube method, when these amounts of serum are added to 2 c.c. of antigen (turbidity 7 cm. by Gates apparatus), the following respective approximate dilutions are obtained:

1–25, 1–50, 1–100, 1–200, and 1–500.

The antigen has been standardized so that the titer of the agglutinins in a given serum will be parallel in the two methods. Therefore, in the rapid test, complete agglutination with the various amounts of serum represents the following titers: 0.08 c.c., 1–25; 0.04 c.c., 1–50; 0.02 c.c., 1–100; 0.01 c.c., 1–200; 0.005 c.c., 1–400.

If one desires to employ quantities of serum different from those just mentioned it will be necessary to standardize the antigen for the amounts in question. It is important to acquire an accurate knowledge of reading the test. If possible, a visit should be made to a laboratory conducting the test to obtain assistance in reading and interpreting the different degrees of agglutination.

The interpretation of the different degrees of agglutination in terms of infection is still a debatable question. One should conform to the interpretation placed on the test by the regulatory or sanitary authorities of the state in which the test is conducted.

The presence of infection in the udder is indicated by complete flocculence of the antigen with 0.04 c.c. of milk serum. In the case of a composite milk sample, infection is indicated when flocculence occurs with 0.08 c.c. of serum.

Differentiation of the Species of Brucella—It is important that one be thoroughly familiar with the peculiar growth characteristics of the Brucella group and the methods that have been successfully used in their differentiation, before an attempt is made to identify the species of a newly isolated strain. It is advisable for those laboratories which isolate cultures at wide intervals to forward new cultures for their identification to laboratory workers who have had considerable experience with the Brucella group.

There are five methods that have been used successfully with certain limitations, for the identification of the species of Brucella. The agglutinin-absorption method was used first by Feusier and Meyer [9] for this purpose. It was studied more exhaustively by Evans [10] and by Wilson and Miles.[11] It is a valuable method for distinguishing *Brucella abortus* from *Br. melitensis* if the technic employed by Wilson and Miles [11] is followed, otherwise it will fail to distinguish one from the other in certain instances. The agglutinin-absorption method is not a reliable one for distinguishing rough strains of melitensis from rough strains of abortus nor will it separate *Br. suis* from *Br. abortus*.

If one is desirous of using the agglutinin-absorption method in determining whether a strain belongs to the abortus or melitensis group, it is recommended that the technic of Wilson and Miles [11] be followed.

The glucose utilization method, first applied and recommended by McAlpine and Slanetz [12] for identifying the species of Brucella, has not found general acceptance by many other workers.

The difference in the nitrate and nitrite reducing ability of the species of Brucella has been made use of by Zobell and Meyer [13] as a means of distinguishing one from the other. Their results obtained by this method have been confirmed by the writer. The method should be employed, wherever possible, in determining the species of a newly isolated culture.

The difference in the hydrogen sulphide metabolism of the three species of Brucella has been made use of by the writer [1] in distinguishing one species from the other regardless of their source or whether the culture is smooth or rough. There is one group of strains the writer and others have studied which cannot easily be identified by the H_2S metabolism method.

They are the Danish strains of *Br. suis*. These strains do not produce H_2S to the same degree that other strains of *Br. suis* produce.

A method [1] which makes use of the difference in growth behavior of the species of Brucella toward certain aniline dyes in a solid medium has been generally accepted as the most satisfactory for identifying the species to which a given culture belongs.

The following technic should be followed in using the dye plate method for identifying the species of Brucella: The medium used is prepared according to the formula given at the end of this section. The pH is adjusted to 6.6. The adjustment should be made just before the dyes are added, as liver infusion medium does not always remain at the pH to which it was previously adjusted if it is heated afterward. This point should be observed closely if successful differentiation of the species is to be obtained. A medium of higher or lower pH gives such confusing results that perfect differentiation is impossible.

The dyes which have been found to give consistent results in the differentiation of the three species are thionin and basic fuchsin. They are certified dyes, made by the National Aniline and Chemical Company.

The dyes are prepared in a 1 per cent stock solution or suspension in sterile distilled water. They are not readily soluble in water in this concentration. The stock solutions appear to keep indefinitely. The final dilution which should obtain for thionin is 1:50,000 and for basic fuchsin 1:25,000. These dilutions are based on the actual amount of the original dye in the medium.

The dye suspensions should be heated in flowing steam for 20 minutes, shaken well and, while still hot, added to the melted medium before it has time to cool. This procedure results in a more uniform mixture of the dye suspension and a more uniform distribution of the dyes in the medium. The medium and dyes are thoroughly mixed and immediately poured into Petri plates. The plates are placed in a 37° C. incubator until the water of condensation disappears.

The plates may be divided into three or more sections to accommodate the growth of several strains of the organism. The seeding of the plates is accomplished with a loop of a heavy suspension of a 48 to 72 hour agar slant growth. The suspension may be obtained by working up a portion of the growth in the water of condensation at the butt of the slant or by adding a small amount of sterile broth or sterile saline solution to the slant. It may be stated here that the dyes do not kill the organism, but merely inhibit its reproductive function; so if masses of the culture are streaked over the surface of the plates, slight growth is very apt to occur at those points where the seeded mass has not been evenly distributed.

The seeded plates are incubated aerobically at 37° C. for 72 hours, or in 10 per cent CO_2 when newly isolated bovine strains are used. At the end of the period of incubation one will find that strains of the abortus species have grown only on the medium containing basic fuchsin; those of the suis species have grown only on the medium containing thionin; and those of the melitensis species have grown on each of the media. The growth of the last species, however, is as a rule never as luxuriant as that of the other two species.

PREPARATION OF LIVER AGAR MEDIUM [1]

The medium is prepared as follows: Fresh beef liver, free from fat, is ground in a meat chopper to a plastic mass. One pound of the liver and 500 c.c. of tap water are placed in a con-

tainer and mixed well. The container is covered and placed in flowing steam for 20 minutes. Remove the lid and stir with a glass rod in order to mix thoroughly so that all parts are reached by the heat. The heating is continued in flowing steam for 1½ hours. Remove and filter through a wire screen. The infusion thus prepared is made into a solid medium or infusion broth.

To prepare one liter of liver infusion agar, measure out the following ingredients:

Washed agar	20 gm.
Tap water	500 c.c.
Liver infusion	500 c.c.
Peptone (Bacto)	10 gm.
Sodium chloride C.P.	5 gm.

Place all ingredients in a suitable container, cover and place in flowing steam for 1 hour. Remove and cool to 60° C. Adjust the pH at this time to 7. Place in flowing steam again for ½ hour. Decant and place in sterile flasks or tubes and sterilize at 15 lb. pressure for 30 minutes. The reaction of the medium during the process of sterilizing will usually drop to or near pH of 6.6. In the writer's experience the organisms grow best at or near pH of 6.6. The final medium prepared according to the foregoing method will not be clear, but this will not interfere with its use as a medium. The final product may be freed from sediment and suspended particles if it is passed through a Sharples centrifuge before sterilization.

Liver broth for blood culture or other use may be prepared in the same way.

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I. FOREST HUDDLESON,
Associate Referee

Examination of Water and Sewage*

THE committee this year offers definite recommendations for changes for the Eighth Edition of *Standard Methods for the Examination of Water and Sewage*. While the definite proposals will be recommended for action in a later business meeting of the Laboratory Section, it seems desirable to present before this meeting some discussion of these specific changes, together with more general considerations, which, although of an editorial character, should be brought to the attention of those interested in water and sewage analysis. Most of the proposed changes were anticipated in the report presented in 1934.

A rearrangement of material in the various Parts of the book is made necessary by the inclusion of more complete procedures for sewage analysis and for boiler water examination, in order to place the methods in the sections to which they are most applicable and to avoid repetition.

Part I will contain the procedures most frequently used in sanitary chemical analysis.

Part II will include methods more often used in mineral analysis, together with the new section on boiler water analysis, and the present section containing rapid methods which are used primarily in railroad laboratories. It is of course impossible to avoid some arbitrary decisions in assigning the procedures on this basis.

The methods for dissolved oxygen and oxygen consumed have been transferred to Part III which deals with sewages and sewage effluents and has been prepared by a committee from the Sewage Works Association. Part IV contains methods for the analysis of sludge, etc.

Parts V and VI deal with microscopic analysis, and Part VII includes the bacteriological methods. Certain procedures now contain rather rigid specifications for laboratory apparatus. It has seemed convenient to gather this material in one section.

The more important changes in methods will be discussed.

The determination of odor has been completely revised and now includes the so-called "threshold" method, embodying the ideas of Spalding as recommended by Fair and his associates, and an optional air dilution test.

More discussion has been aroused by the present procedure for estimation of chlorine than by any other of the chemical analyses. This has led to a complete revision of the procedure and its modification to allow for the numerous interfering substances. In the presence of relatively large amounts of nitrite nitrogen and manganic compounds the ortho-tolidine method is unusable, so that an iodine procedure has been added.

In the western part of the country it is sometimes necessary to determine the borate content of a water. The method now in Appendix II has been used in extensive studies by the Los Angeles Water Department and found sufficiently satisfactory to warrant the recommendation that it be included as a standard procedure. Two other determinations should also be similarly transferred: The nephelometric method for zinc, and the DeBoer method for fluorides by the use of the zirconium-alizarin reagent.

* Report of the Standard Methods Committee.

For some time the various committees in charge of revising Standard Methods have hoped to include more accurate procedures applicable to boiler water analysis. This section has been prepared under the direction of S. T. Powell and includes the methods recommended by the American Society for Testing Materials.

The procedure for microscopic methods has been revised and a table has been placed in the Appendix to give suggestions for interpretation of results.

In the paper presented by Mr. Ruchhoft, the basis for changes in bacteriological methods has been discussed. The large amount of laboratory data involved has been obtained in a coöperative investigation under Mr. Ruchhoft's direction, and the committee is highly appreciative of the interest and coöperation of the more than 20 laboratories involved. Various media were studied on the basis of their growth promoting properties for the coli-aerogenes group and their inhibitive action toward anaerobes. Much of the latter work was done by Dr. R. S. Spray. From the data obtained, certain media were selected for use in a confirmatory test for the coli-aerogenes group. None of the special media studied were considered satisfactory to supplant lactose broth for primary inoculation with portions of water samples but are satisfactory for confirmation. It is hoped that this new procedure will materially shorten the present standard method and will give results equally satisfactory from a sanitary standpoint.

The entire section on bacteriological methods has been carefully revised, and a new schematic outline prepared. The most important changes in these methods are those dealing with the detection of organisms of the coli-aerogenes group. It is proposed to recognize 3 tests—presumptive, confirmed, and completed. No change is recommended in the presumptive test except that tubes showing gas after 48 hours should be confirmed rather than completed. The confirmed test may be done either by the use of Endo or eosin-methylene blue plates, as at present, or by the use of one of 4 confirmatory media:

Brilliant Green Bile
Crystal Violet Lactose Broth
Fuchsin Lactose Broth, or
Formate Ricinoleate Broth

The formation of gas in any one of these media following the formation of gas in a lactose broth tube will constitute the confirmed test. No change has been made in the completed test except that completion may be made from a confirmatory medium as well as from Endo or eosin-methylene blue plate. It is desirable, but not required, in selecting the confirmatory medium that the medium be studied in relation to the particular water supply under examination, and that the one be chosen which gives the most satisfactory correlation with the completed test.

One addition has been made to the committee since last year. Dr. W. L. Mallmann has been added as a member in charge of methods for swimming pool examination. In general, these methods would follow those of sanitary water analysis, but it is well known that certain precautions must be taken in collecting samples from chlorinated pools. Inasmuch as Dr. Mallmann is the Laboratory Section representative on a committee of the Public Health Engineering Section, it is not possible to include his report in the main body of the text of *Standard Methods* until a report is approved by the committee. However, his suggestions will be included as an Appendix to *Standard Methods*.

Below are the definite recommendations of the committee:

Proposed Recommendations for Presentation to the Laboratory Section by the Coördinating Committee and Relating to the Eighth Edition of Standard Methods for the Examination of Water and Sewage

AS in the past, these recommendations include only proposed changes which are fundamental in character, *i.e.*, new methods which the committee recommends for inclusion in the Eighth Edition and fundamental changes in present methods. Since the new manuscript must be approved, both by the Coördinating Committee and the Committee on Research and Standards, it is believed that sufficient safeguards exist over proposed changes of an editorial character.

The following changes and additions are recommended:

1. The addition of a section dealing with laboratory apparatus, this section being a compilation of specifications already included in the text under various methods of analysis.

2. The use of milliequivalents per liter in figuring chemical constituents in addition to parts per million.

3. The so-called "threshold" method for detecting odors in water, essentially that proposed by Fair. The air dilution method is an optional procedure. This involves the use of more descriptive terms than at present employed, together with the use of code letters.

4. A revision of the test for chlorine, involving a modification of the time allowed for color development. This will be not less than 5 minutes and not more than 15 minutes, with the provision that a less time may be used under special circumstances. Where interfering substances are present, procedures will be given in the appendix.

5. The carbamate method for the examination of copper in place of the electrolytic procedure.

6. The deletion of the present method for tin.

7. The method for estimating zinc now given in the Appendix (nephelometric ferrocyanide method).

8. The method for boron now described in the Appendix (electrometric).

9. The DeBoer Method for estimating fluoride (colorimetric with zirconium-alizarin reagent).

10. Perchloric acid method for potassium.

11. The method for determination of iodide now given in the Appendix (colorimetric with nitrosyl-sulphuric acid reagent).

12. The elimination of the method for oxygen consumed in Part I of the book and the transfer of this procedure to Part III dealing with sewage analysis, leaving an appropriate cross-reference.

13. Transfer of the determination for dissolved oxygen from the section dealing with water analysis to the section dealing with sewage analysis with appropriate cross-reference.

14. A determination for total solids in brines. Drying at 750° C.

15. Permission to use other than specified indicators in alkalinity.

16. Addition of a colorimetric method for silica (ammonium molybdate).

17. Phosphate method now in Appendix (precipitation as phosphormolybdate and as magnesium ammonium phosphate with ignition—Scott method).

18. A section entitled, "Analysis of Stationary Boiler Water Supplies"; this section to include the following new determinations:

Strontium chloride method for hydroxide.

Barium chloride and carbon dioxide evolution methods for carbonate.

Benzidine and tetrahydroxyquinone methods for sulphate.

Colorimetric method for phosphates (aminonaphtholsulfonic acid).

Dissolved oxygen—a modification of the Winkler procedure to detect small amounts of oxygen.

Sulphite (iodine titration).

Cross-references have been used for methods in other sections of the book.

19. The adoption of the methods for sewage analysis, including sewage sludge and muds, as proposed by a committee of the Federation of Sewage Works Associations and published in the *Sewage Works Journal* for May, 1935. These methods will be edited only sufficiently to conform to the rest of the text. They do not include any essentially new items—except that for chlorine demand—but represent, rather, modifications of present procedures. Where possible, references are made to other portions of the text rather than repeating complete procedures elsewhere described.

20. The inclusion in the section on sewage analysis of the complete procedures for the determinations for oxygen consumed and dissolved oxygen.

21. In the microscopic method, complete description of the use of the centrifuge for concentration is included. It is proposed to include in the Appendix of the volume a table with suggestions for the interpretation of results.

22. Dilution water for use in the bacteriological procedures may be either tap water or one of the dilution waters recommended in the test for biochemical oxygen demand.

23. In the bacteriological methods it is proposed to set up 3 tests for the detection of the coli-aerogenes group:

Presumptive Test
Confirmed Test
Completed Test

The presumptive and completed tests are essentially those in the present edition. In the confirmed test, it is proposed to allow the use of Endo or eosin-methylene blue agar plates or to confirm in one of four specified liquid media—confirmation to be complete when gas appears within 48 hours in the confirmatory medium inoculated from tubes showing gas as a result of primary planting in lactose broth. These media are:

Brilliant Green Bile
Crystal Violet Lactose Broth
Fuchsin Lactose Broth
Formate Ricinoleate Broth

24. Completed tests may be made either from lactose broth tubes showing gas or from tubes of a confirmatory medium showing gas.

25. Parallel planting in lactose broth and brilliant green bile for control in water purification plants be removed from the text and placed in an Appendix.

26. The formula for the preparation of Endo media has been revised in accordance with the work of Conn in coöperation with the members of the committee. The essential feature of this is a more definite statement in regard to the stock solution of fuchsin. The proposed strength is a 3 per cent solution. The present optional formula for Endo medium will be placed on a par with the first formula.

27. The method for calculating the coli-aerogenes group density, known as the Most Probable Number. The present method of calculating this index is retained as an alternative procedure for the use of single samples of water and will be known as the Indicated Number.

While the above constitute the definite fundamental changes recommended by the committee, it is desirable that the section be informed concerning certain proposed rearrangements of an editorial character. Some of these rearrangements have been mentioned above because of fundamental importance. Others include the division of Part I into two parts, the first to contain methods essential to or used in the sanitary examination of water, and the second part to contain the procedures dealing with mineral analyses. While it is recognized that such a division must be somewhat arbitrary because certain determinations might be included in either section, it is believed that this division will facilitate the use of the book. A new system of numbering the paragraphs will be used with the hope that cross-references will be less complicated than at present. This consists of a modification of a decimal numbering system. Certain of the procedures have been rewritten because of criticisms received from numerous sources. For example, the method for the determination of chlorine has been entirely revamped, as have the sections on turbidity, hardness, microscopic methods, and bacteriological procedures. In the latter, a new schematic arrangement to include the methods for the detection of the coli-aerogenes group has been devised and this will replace two of the present

sections which were found to be more or less cumbersome. The "outline of the daily routine" has been deleted. In connection with the newly recommended confirmatory liquid media, it has, of course, been necessary to include the method of preparation of these media. The present statement concerning variations in media when large samples of water are used for examination, has been clarified.

JOHN F. NORTON, *Chairman*
A. M. BUSWELL
F. E. DANIELS
W. L. MALLMANN
THEODORE A. OLSON
C. C. RUCHHOFT
WILLEM RUDOLFS

Diagnostic Procedures and Reagents

THE Section on Diagnostic Methods and Reagents of the Standard Methods Committee of the Laboratory Section has now been working for 2 years. Only one new referee, Dr. Henry P. Welch, referee for typhus fever, has been appointed since the last report. The referees and the subjects they are investigating are named in the *Year Book* for 1934–1935. In that report there also appear papers by several of the referees which are preliminary reports on the formulation of what has been called standard laboratory methods for the diagnosis of certain communicable diseases. In these reports it is clear that neither the committee nor any referee has approached the problem with the idea that methods will be devised which are final and unchangeable. It is well understood by the committee that anything which we report can be looked upon only as recommended methods and what we consider to be the best technic in view of our present knowledge to demonstrate characteristic biological features of certain microorganisms. Often during discussions of the desirability of the description of such methods objection is raised to the use of the phrase: "Standard Methods." The objection is always based upon the idea that there is implied by this phrase methods which are fixed and unchangeable, and that the Association is forever committed to them as the final opinion upon a completed subject.

Such controversies seem to show a complete failure to comprehend the purpose of recommending methods of procedure. It is, of course, apparent to all that the environment which we create for microörganisms determines what their reaction will be. Therefore any new knowledge concerning the influence of environment on their behavior may at once modify the technic used for the demonstration of their biological reactions. With an understanding of the shortcomings of our knowledge concerning the influence of environment upon the behavior of pathogenic microörganisms, and the behavior of these organisms to different environmental conditions, it is readily conceded that a final word on the methods to be employed in their study cannot now be said. But, on the other hand, the very uncertainty of our knowledge upon these questions makes more certain the importance of uniform methods of study. It needs no argument to show that if one laboratory attempts the isolation of *B. typhosus* from stools preserved in a special preservative, no comparison with the results of another laboratory which does not use such a preservative is possible. It is not alone for the purpose of comparison, however, that uniformity of method is desirable. Since the environment created by one laboratory is often so different from that of another, it is quite likely that one method gives better results than another or in certain instances a method may be entirely unsuited to the purpose for which it is used. A Standard Method, or recommended method, would give a uniformity growing out of a wide experience of many laboratories and out of the experience of large laboratories where time and money have made possible special investigation. Such methods, while not representing the ultimate of perfect knowledge, certainly do offer

an opportunity for the temporary or tentative standardization of methods.

In a discussion of the committee's conception of its task, there is another phase of laboratory work which should be mentioned, *i.e.*, the interpretation of laboratory results in terms of medical diagnosis. This phase of the work is influenced by so many factors which are not subject to methodology that no standard significance can be arbitrarily applied to any given laboratory results. Some of the factors which influence interpretations are the method by which the specimen is collected and how it is preserved, but at the juncture where the results of laboratory and clinical studies are put together is the point where standardization ends and where failure so often occurs. Failure results sometimes because the pathologist has not been supplied with sufficient clinical information upon which to base an interpretation of his finding. A contributing cause to such failure is due often to erroneous conceptions concerning the significance of laboratory findings. By some they are considered to be mathematically exact. No one knows better than the trained laboratory worker that such is not the case. There is no single laboratory determination which can be removed from the clinical environment which it was devised to elucidate, and its clinical significance evaluated. Thus the finding of morphological *B. diphtheriae* in a throat culture does not diagnose clinical diphtheria nor does a positive agglutination test diagnose typhoid fever, undulant fever, or other diseases, etc.

With all of these limitations in mind the Committee on Diagnostic Procedures and Reagents began its work of investigating the possibility of formulating methods which could more or less standardize certain phases of laboratory procedure. The first year was spent in selecting and appointing referees and in gathering data through questionnaires and by other means from laboratories throughout this country. The reports of these studies, those that have been completed, will be found in the *Year Book* and papers published in the *Journal* during 1935. The last year has been spent by the referees in the formulation of completed manuscripts describing methods of procedure for the recognition of infections with the various pathogenic microörganisms.

Because manuscripts from all of the referees have not been completed, and because those which are completed and ready for publication will undoubtedly be revised from time to time as information accumulates, it is recommended that the manuscripts which we now have be published in a loose leaf volume, and made available at a reasonable cost to laboratories wishing to use them. We now have 11 completed manuscripts, and there is another almost completed. The volume will therefore contain a description of methods used in the recognition of the following conditions and microörganisms.

1. Studies on the Toxicity of Brilliant Green for Certain Bacteria
2. Serological Tests for the Diagnosis of Syphilis
3. Serological, Bacteriological, and Other Biological Procedures in the Diagnosis of Undulant Fever
4. Recognition and Significance of Hemolytic Streptococci in Infectious Diseases
5. Serological and Bacteriological Procedures in the Diagnosis of Enteric Fevers
6. Whooping Cough and *B. pertussis*
7. Laboratory Diagnostic Procedures in the Recognition of Various Food Poisonings
8. Meningitis and Meningococcus
9. Gonorrhea and the Gonococcus
10. Amebiasis and *Ameba Histolytica*
11. Tuberculosis and the Tubercle Bacillus

Manuscripts which have been previously published in the *Year Book* but have been revised and manuscripts which have not been previously published are submitted with this report.

W. D. STOVALL, *Chairman*

Tentative Methods for the Diagnosis of Amebiasis and Amebic Dysentery*

I. Definition
II. Symptomatology
III. Etiology
IV. Differential characteristic of the Intestinal Amebae of man
  B. *E. histolytica*
    a. Trophozoites
    b. Precysts
    c. Cysts
  C. *E. coli*
    a. Trophozoites
    b. Precysts
    c. Cysts
  D. *Endolimax nana*
    a. Trophozoites
    b. Precysts
    c. Cysts
  E. *Iodameba bütschlii*
    a. Trophozoites
    b. Precysts
    c. Cysts
  F. *Dientameba fragilis*
V. Diagnostic Procedures
  A. Character of stool and collection of specimen
  B. Examination
    a. Active cases
    b. Convalescent cases, and carriers
    c. Pus from liver and lung abscesses
    d. Procedures for fixing and staining
    e. Concentrating stool for cysts
    f. Cultural methods
    g. Complement fixation
  C. Illustrations
VI. Microscopic Examination
VII. Examination of Stools from Convalescent Cases and Carriers
VIII. Examination of Pus from Liver and Lung Abscesses
IX. Concentrating Stools for Cysts
X. Cultural Methods
XI. Complement-Fixation in the Diagnosis of Amebiasis and Amebic Dysentery

I. Definition

"*Amebiasis* is the state of being infected with amebae. In practice it refers to *E. histolytica*." Dobell.

"*Amebiasis* is the invasion of the tissues of man by *E. histolytica*. The invasion is primarily through the mucous membrane of the large bowel—much more rarely through the mucous membrane of the ileum.

"*Amebic Dysentery* is a bloody mucoid diarrhea caused by *E. histolytica* and occurring as one of the manifestations of amebiasis." Craig.

II. Symptomatology

The symptoms vary from slight digestive disturbances to the most severe symptoms of amebic dysentery or amebic abscesses of the liver or other organs. In some individuals the symptoms are mild with few stools a day, while in others the symptoms are very severe—there is a sudden onset, with headache, severe colicky pain in the abdomen, malaise, tired, anxious, and drawn expression, tongue dry and furred, tenesmus and desire to defecate. There may be 6–30 bloody mucoid stools a day; emaciation, prostration and death from cardiac failure and exhaustion.

III. Etiology (*Endameba histolytica*)

Among the various amebae in man are:

* Report of the Referee on Amebiasis for the Standard Methods Committee on Diagnostic Procedures and Reagents.

Genus I. *Endameba* (Leidy, 1879)
Species in man—
E. histolytica, Schaudinn, 1903
E. coli, Grassi, 1879
E. gingivalis, Gros, 1849

Genus II. *Endolimax* (Kuenen and Schwellengrebel)
Species in man—
Endolimax nana, Wenyon and O'Connor, 1917

Genus III. *Iodameba* (Dobell, 1919)
Species in man—
Iodameba bütschlii

Genus IV. *Dientameba* (Jepps and Dobell, 1918)
Species in man—
Dientameba fragilis

IV. Differential Characteristics of the Intestinal Amebae of Man

The main criterion upon which the differences are based is largely the nuclear structure which involves the thickness of the nuclear membrane, size, type, and position of the karyosome, linin network (delicately stained fibrils in spaces between the karyosome and the nuclear wall), and chromatin granules. In addition to the nuclear structure, various other features are important—the number and type of nuclei, the type of chromatoid bodies, and the glycogen.

B. *E. Histolytica*

E. histolytica is divided into different races according to the size of cysts produced (Plates I and II).

Trophozoite—15–80 micron.

E. histolytica which produce small variety cysts are usually smaller and not quite as rapidly motile as those producing large variety cysts. In the cases which we have had we have never been able to find red blood cells in the trophozoites which gave rise to small variety cysts. Bloody mucous stools from acute cases of amebic dysentery usually show quite large *E. histolytica* trophozoites; whereas the trophozoites found in soft stools from mild cases of amebic dysentery are usually smaller in size approaching in size those which give rise to small variety *E. histolytica* cysts.

Cytologically the trophozoites have clear, glass-like ectoplasm and finely granular endoplasm. The motility is usually in a straight line by means of a single large blade-like pseudopodium which moves rapidly and explosively. Usually the nucleus flows into the pseudopodium first, the red blood cells and the finely granular material follow the nucleus into the pseudopodium. In fresh, bloody mucous stools (from acute cases of amebic dysentery) the motility is very rapid. In old stools the motility is more sluggish and the pseudopodia are blunt blade-like.

Precysts—Before encystment the organism becomes round or slightly oval, hyaline (by ridding itself of ingested material), smaller in size, and loses motility completely or it may continue to send out very small blunt pseudopodia. The nucleus consists of a ring of small refractile granules. The precyst secretes a delicate wall and encysts.

Cystic stage—Size 5–20 μ (small variety, 5–12 μ; large variety, 12–20 μ)—The cysts contain 1–4 nuclei, although as many as 8 have been described. The nuclei have characteristic structures—thin nuclear membrane composed of fine granules of chromatin and a very small centrally placed karyosome.

Young cysts have typical chromatoid bodies—rods with blunt ends. These show up best in moist preparations. In iodine stained preparations, brown staining glycogen may be seen. Both the chromatoid bodies and the glycogen disappear when the cysts are old.

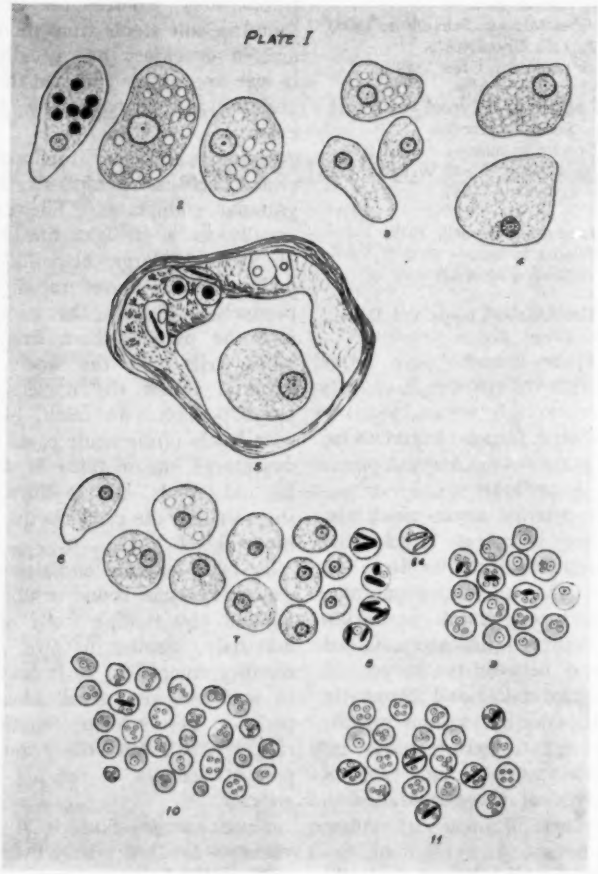

PLATE I, Figures 1–11—*E. histolytica* (Large variety, except for few small cysts, Fig. 10)

Fig. 1. Trophozoite, showing the nucleus and red blood cells (from a bloody mucous stool of an active case, M.) (Red blood cells, chromatin granules and karyosome filled in with India ink to show up better)

Fig. 2. Trophozoites from an iron-hematoxylin preparation

Fig. 3. Trophozoites from a section of an amebic ulcer of the large bowel (iron-hematoxylin stain)

Fig. 4. Trophozoites from a section of a liver abscess (iron-hematoxylin stain)

Fig. 5. A trophozoite in a capillary of the large bowel (from the same section as Fig. 3)

Fig. 6. Trophozoite passing into the precystic stage (water preparation)

Fig. 7. Precysts of various sizes (water preparation)

Fig. 8. Young cysts (water preparation) showing typical chromatoid bodies and nuclei. (Figs. 6, 7, and 8 are from case M. M.)

Fig. 8a. Chromatoid bodies and nucleus as seen in water preparation.

Fig. 9. *E. histolytica* cysts, large variety (iodine preparation) showing nuclei with karyosomes, chromatoid bodies and glycogen (same case as Figs. 6, 7 and 8)

Fig. 10. Large, intermediate, and small *E. histolytica* cysts. case C. N. (iodine preparation)

Fig. 11. *E. histolytica* cysts—large variety, case R (iodine preparation)

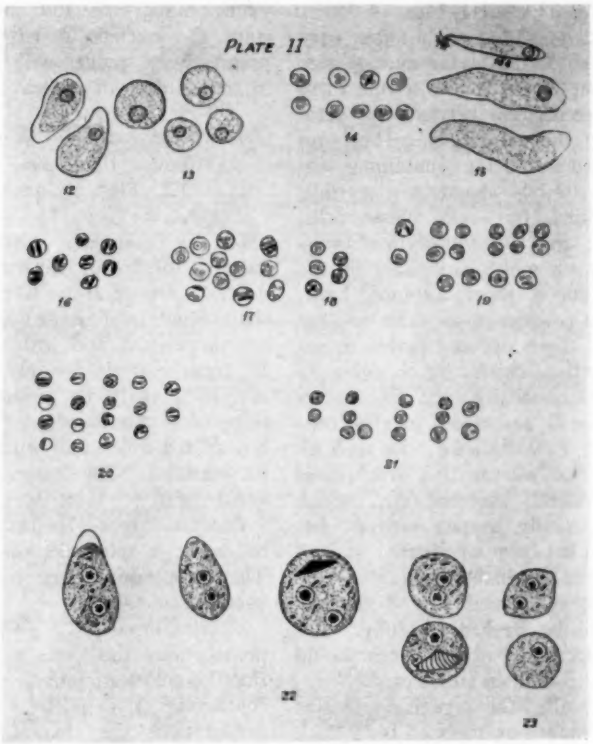

PLATE II. Figures 12–21—*E. histolytica*, small variety

Fig. 12. Trophozoites showing nucleus (water preparation)
Fig. 13. Precysts (water preparation)
Fig. 14. Cysts (iodine preparation)
Fig. 15. Trophozoites from a 24 hr. culture (case H. Sch. same as Figs. 12, 13 and 14)
Fig. 15a. Trophozoite from a 24 hr. culture from Case R.
Fig. 16. Small and intermediate cysts (water preparation) showing chromatoid bodies and nuclei (case J. R. G.)
Fig. 17. Intermediate sized *E. histolytica* cysts (iodine preparation) same as case in Fig. 16)
Fig. 18. Small *E. histolytica* cysts (iodine preparation) same case as 16 and 17
Fig. 19. Small *E. histolytica* cysts (iodine preparation) from case Wm. L. J.
Fig. 20. Small *E. histolytica* cysts (water preparation) from same case as Fig. 19
Fig. 21. Very small cysts of *E. histolytica* (iodine preparation) same case as Figs. 19 and 20)

Figs. 22 and 23—Dientameba fragilis:
Fig. 22. Trophozoites (saline preparation) from case K.
Fig. 23. Trophozoites from iron hematoxylin preparations brought from Hamburg

NOTES EXPLAINING THE DRAWINGS

Most of the drawings were made from moist preparations (saline, water, and iodine) of fresh material, with a camera lucida, using a number 10 eye piece and a high, dry, 1/6" objective. (Bacteria, crystals, yeast cells, starch grains, red blood cells, karyosomes, chromatoid bodies, and chromatin granules have been filled in with India ink in order that they may show up better.) The oil immersion lens was used in making drawings from sections of tissue and from iron-hematoxylin preparations.

C. *E. Coli* (Plate III, Figs. 24–30)

Trophozoite—The trophozoites vary in size from 15–50 μ, the average size being about 20–25 μ. As a rule there is no differentiation between the ectoplasm and the endoplasm. There are many food vacuoles containing ingested material — bacteria, crystals, starch grains, pus cells, yeast cells, flagellates, and *E. histolytica* cysts. The organism moves sluggishly in one direction for a short distance, halts, produces a pseudopodium from another portion of the body, and moves in another direction, chiefly by changing its shape. Occasionally one encounters very motile *E. coli* which may be confused with *E. histolytica*. In such an event careful examination will reveal ingested debris, bacteria, etc., which does not usually happen with *E. histolytica*. In case of doubt, stained preparations should be made, using the iron-hematoxylin method and the nuclei should be studied carefully. Repeated direct stool examinations should be made, as well as cultures.

Cytologically, the organism contains a visible nucleus surrounded by a thick membrane composed of coarse chromatin granules. The karyosome is fairly large and usually to one side of the center. Chromatin granules are present in the clear zone between the karyosome and the nuclear membrane.

Precystic stage—15–30 μ—In this stage it is frequently difficult to differentiate *E. histolytica* from *E. coli*. As a rule, however, *E. coli* has a thicker wall, the cytoplasm is more granular, and the chromatin granules are coarser.

Cystic stage—10–30 μ (average size 14–20 μ)—The cysts of *E. coli* have fairly thick walls. There are 1–8 nuclei (16–32 have been described). We have seen 12–16 nuclear cysts. The karyosome is usually large and eccentric. The chromatoid bodies are needle- or splinter-like, with sharply pointed ends. Glycogen is present in young cysts. In the single nuclear stage the nucleus is large, becoming progressively smaller with the increase in the number of nuclei.

D. *Endolimax nana* Wenyon and O'Connor, 1917 (1918) (Plate III, Figs. 36 and 37)

Trophozoite — 8–15 μ — The cytoplasm is granular, containing food vacuoles filled with bacteria, yeasts and debris. The motility is by means of short, blunt, hyaline pseudopodia, which are thrown out and withdrawn slowly. In fresh material or in cultures the motility may be by means of slender, finger-like pseudopodia. The nucleus has an indistinct wall without chromatin granules. The karyosome is large and irregular.

Precystic stage—In unstained preparations it is refractile, round, or oval. The cytoplasm is free from vacuoles, bacteria, and debris.

Cystic stage—5–12 μ—In unstained preparations the cysts appear as refractile, colorless, round or oval bodies containing 1–4 nuclei. In stained preparations the characteristics stand out plainly—there are 1–4 nuclei with faint nuclear membranes and no chromatin granules. There is a large central or eccentric karyosome in each nucleus. Glycogen is rarely seen.

E. *Iodameba bütschlii* von Prowazek, 1912; Dobell, 1919 (Plate III, Figs. 31–35)

Trophozoite—5–20 μ or larger (average 10–15 μ)—It is a small ameba moving sluggishly, as a rule, by means of broad, round, and hyaline pseudopodia. In living trophozoites the nucleus is frequently invisible. In well stained preparations, however, the small nucleus (2–3.5 μ) with its large central karyosome, shows up well. The nuclear membrane is usually achromatic. Between the karyosome and the nuclear membrane is a zone filled with gran-

IMPORTANT CRITERIA IN DIFFERENTIATING INTESTINAL AMEBAE

	E. histolytica	*E. coli*	*Endolimax nana*	*Iodameba bütschlii*	*Dientameba fragilis*
Trophozoite					
Size	18–60 μ 20–35 μ aver.	20–30 μ 15–20 average	6–12 μ 8 average	5–20 μ 10–15 average	10–50 μ
Motility	Active - progressive directional	Sluggish, progressive, not directional	Sluggish, progressive	Sluggish	Active, progressive
Pseudopodia	Finger - shaped, clear, glass-like	Shorter, more blunt, less glass-like	Broad, blunt, not glasslike	Broad, rounded, hyaline	Flat, leaf-like, hyaline
Inclusions	Red blood cells when stools contain blood. No bacteria in fresh stools	No red blood cells. Bacteria, yeasts, crystals, cysts, etc.	No red blood cells, bacteria, debris, etc.	Bacteria, debris, etc.	Bacteria, crystals, debris, etc.
Nucleus	May be invisible	Visible	Visible	Freq. invisible in unstained preparations	Usually visible
Nuclear membrane	Delicate, with single layer of minute chromatin granules in inner surface	Thicker, inner surface lined with coarser chromatin granules	Thin, chromatin rarely seen in inner surface	Thin, but thicker than in *E. histolytica*	Thin, delicate and free from chromatin except in degenerating forms
Karyosome					
Size	Very small; usually in center of nucleus	Larger, usually eccentric	Large, and may be divided into 1 large and 1 small	Large and central	Large, usually composed of 4 or more deeply staining granules
Intranuclear chromatin	No chromatin between karyosome and membrane	Chromatin granules between karyosome and membrane	Mass situated at one side or in center of nucleus	Frequently small granules of chromatin on inner side of membrane. Linin network between karyosome and membrane	
Cystic Stage					
Size	5–20 μ	10–20 μ	5–10 μ	5–20 μ	
Shape	Mostly spherical, may be oval or irregular	Spherical, oval or irregular	Spherical, oval or ellipsoidal	Round, oval, rhomboidal, triangular, fusiform	
Nuclei	1–4	1–8	1–4	1, rarely 2	
Karyosome	Minute, central	Larger, eccentric	Large, central or to one side	Large, eccentric, in contact with membrane (signet ring)	
Nuclear Membrane	Delicate	Thicker	Delicate	Thin	
Chromatoid bodies	Bar, oval or rod-like with blunt ends. Present in about 50% of cysts	Splinter- or needle-like; pointed ends. Present in about 10% of cysts	None	None. "Iodophilic" glycogen mass	

ules. When the preparation is well stained the linin network (between the karyosome and the nuclear membrane) shows up. The cytoplasm is homogeneous and finely granular, containing food vacuoles filled with bacteria, crystals and various debris. The glycogen mass is usually large, and stains dark brown with iodine. In iron hematoxylin preparation the glycogen vacuole can be seen.

Precystic stage—In this stage the organism is free from food vacuoles; it appears clear, glassy white and contains a large nucleus. It rounds up, secretes a cyst wall and encysts.

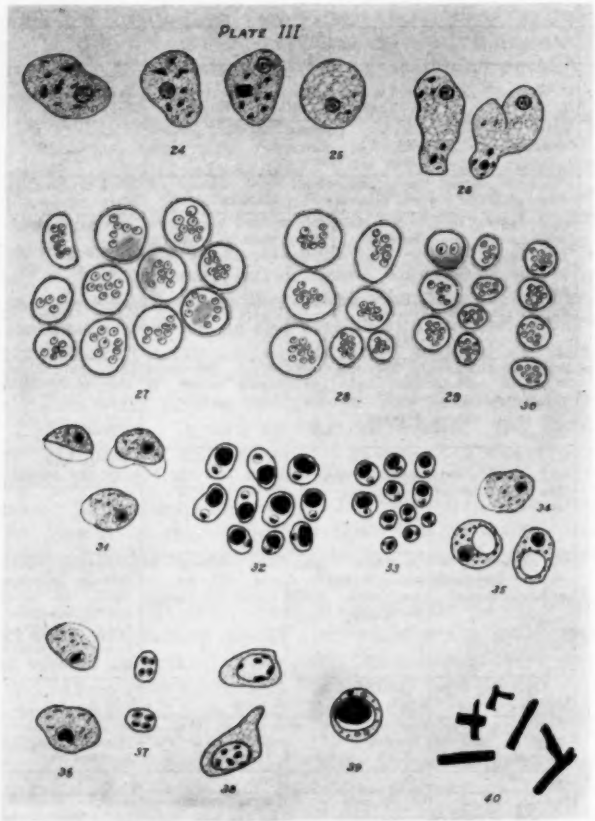

PLATE III, Figures 24–30—*Endameba coli*

Fig. 24. Trophozoites (water preparation) from case E. L.

Fig. 25. Precysts from the same case (water preparation)

Fig. 26. Very motile trophozoites from the same case (water preparation)

Fig. 27. Quite large *E. coli* cysts (iodine preparation) from case Sp.

Fig. 28. *E. coli* cysts of various sizes (iodine preparation) 1 shows 10 nuclei—Case Chas. S.

Fig. 29. *E. coli* cysts (iodine preparation) showing various sizes and various stages of the division of the nuclei

Fig. 30. *E. coli* cysts (iodine preparation) quite uniform in size, from case J. N.

Figs. 31–35—Iodameba bütschlii:

Fig. 31. Trophozoites (water preparation) from case E. L.

Fig. 32. Cysts (iodine preparation) from case J. R. G.

Fig. 33. Cysts (iodine preparation) from case E. C.

Fig. 34. Trophozoite from iron-hematoxylin preparation

Fig. 35. Cysts from iron-hematoxylin preparation, showing the typical nucleus, glycogen vacuole and volutin granules

Figs. 36–37—Endolimax nana:

Fig. 36. Trophozoite, iron-hematoxylin stain

Fig. 37. Cysts, iron-hematoxylin stain

Fig. 38. Macrophages from a section of an amebic ulcer of the bowel

Fig. 39. Blastocystis hominis (iron-hematoxylin preparation)

Fig. 40. Bismuth crystals (water preparation)

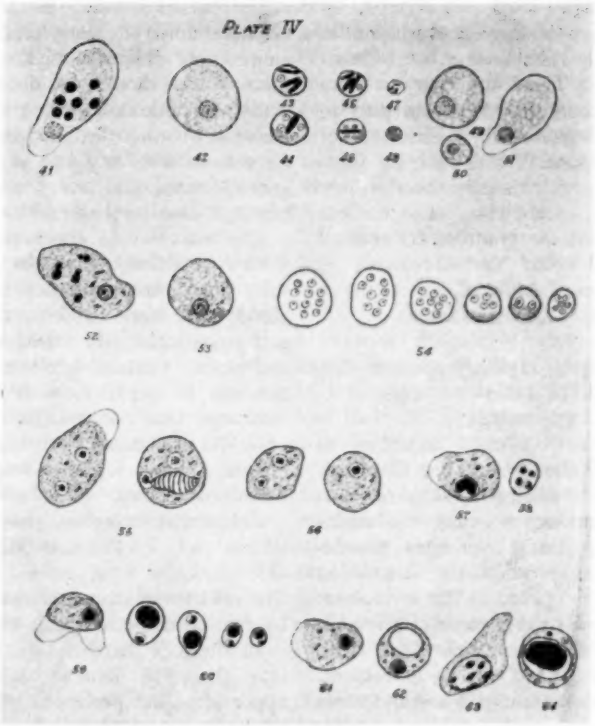

PLATE IV—Summary Plate

Fig. 41. *E. histolytica* trophozoite from a bloody mucous stool

Fig. 42. *E. histolytica* precyst (water preparation)

Fig. 43. *E. histolytica* cyst, large variety (water preparation)

Fig. 44. *E. histolytica* cyst, large variety (iodine preparation)

Fig. 45. *E. histolytica* cyst, intermediate size (water preparation)

Fig. 46. *E. histolytica* cyst, intermediate size (iodine preparation)

Fig. 47. *E. histolytica* cyst, small variety (water preparation)

Fig. 48. *E. histolytica* cyst, small variety (iodine preparation)

Figs. 49, 50 and 51. Large precyst, small precyst, and trophozoite from a specimen showing small variety *E. histolytica* cysts

Figs. 52–54—E. coli:

Fig. 52. Trophozoite (water preparation)

Fig. 53. Precyst (water preparation)

Fig. 54. Cysts of various sizes and number of nuclei (iodine preparation)

Figs. 55 and 56—Dientameba fragilis:

Fig. 55. Trophozoite from saline preparation

Fig. 56. Trophozoite from iron-hematoxylin stain

Figs. 57 and 58—Endolimax nana

Fig. 57. Trophozoite (iron-hematoxylin stain)

Fig. 58. Cysts (iron-hematoxylin stain)

Figs. 59–62—Iodameba bütschlii:

Fig. 59. Trophozoite (water preparation) showing pseudopodia

Fig. 60. Cysts (iodine preparation)

Fig. 61. Trophozoite (iron-hematoxylin stain)

Fig. 62. Cysts (iron-hematoxylin stain) showing nucleus, glycogen, vacuole and volutin granules

Fig. 63. Macrophage from a section of an amebic ulcer of the bowel

Fig. 64. Blastocystis hominis. (It is a vegetable organism, a non-pathogenic fungus, about 5–40μ in diameter. It may be oval or spherical with a narrow band of cytoplasm containing refractile granules and one or more round or oval nuclei surrounded by a delicate membrane. Dividing forms, seen so frequently in cultures, have an hour glass appearance due to the constriction near the center of the cell.)

In hematoxylin stained preparations the narrow band of peripheral cytoplasm is unstained and contains well stained nuclei with large deeply staining karyosomes.

Cystic stage—6–20 μ (average, 9–12 μ)—There is a great variation in shape of the cysts; round, oval rhomboidal, etc. There are one or more glycogen masses staining from pale to mahogany brown with iodine. Other inclusions appear giving staining reactions of volutin. Usually there is only one nucleus, rarely two. The nucleus differs in that the granules are massed at one pole and the karyosome is pressed toward the wall, producing a "signet ring" appearance.

F. Dientameba fragilis Jepps and Dobell, 1918 (Plate II, Figs. 22 and 23)

Trophozoite—Usually described as being small, about 12 μ, but they may be quite large, as large as *E. histolytica*. The organism moves rapidly by means of flattened, leaflike, hyaline pseudopodia. There is differentiation between ecto- and endoplasm. The cytoplasm may be finely or coarsely granular, containing food vacuoles filled with bacteria, yeast cells, starch grains, etc. The most important diagnostic feature is the presence of two nuclei. On dividing, the organism results in two uninucleate individuals, the nuclei of which divide, resulting in binucleate organisms. The nuclei are bounded by fine membranes. The karyosome is usually in the center, but may be at one side of the nucleus. The karyosome is composed of granules, usually only four large, deeply stained, chromatin granules.

V. DIAGNOSTIC PROCEDURE

All stools should be collected in clean, dry containers, free from disinfectants. Oil, bismuth, barium, and medications render the stools undesirable. All stools should be examined as fresh as possible. Soft and bloody mucous stools should be examined immediately or as soon as possible and should never be left over night.

No person should be pronounced negative until 6 stools have been examined on different days. Some of these stools should be obtained normally, and others following the administration of salts. In choosing material for examination, any part of the homogeneous stool and every part of the heterogeneous stool should be examined.

The macroscopic appearance of the stool is significant—whether it is hard, soft, liquid, and whether it contains blood, pus, and cellular exudate. A soft stool, intimately mixed with blood and mucus, containing pus and cellular exudate, is much more indicative of bacillary than of amebic dysentery (F. W. O'Connor), whereas bloody, mucous stools are highly suspicious for amebic dysentery.

Balantidial dysentery due to *Balantidium coli*, a ciliate, is indistinguishable clinically from amebic dysentery. The differential diagnosis must be made by finding the etiological organism.

In alcoholic diarrhea the stools simulate those in amebic dysentery in appearance, but persistent examinations of the stools fail to reveal *E. histolytica* and the patient gets well after several days' rest in bed.

In syphilitic ulcerations of the rectum, the stools may be diarrheal or semi-solid, containing mucus and shreds of tissue. The positive Wassermann test is of diagnostic importance here.

Tuberculous ulceration of the bowel, a common complication of pulmonary tuberculosis, is associated with frequent stools containing mucus and small amounts of blood, except shortly before death, when massive hemorrhages may occur. Finding tubercle bacilli in the stools is of no great importance because a patient with pulmonary tuberculosis may swallow some tubercle bacilli in the sputum.

In chronic mucous colitis there are mucous casts and no blood in the stools. Nonspecific ulcerative colitis, on the

other hand, is associated with frequent stools containing pus, blood, and small amounts of cellular exudate.

In malignant diseases of the bowel the stools are frequent and contain much pus and blood (frequently chocolate in color). The differential diagnosis is based on the laboratory findings, proctoscopic examination, and X-ray findings.

However, whatever the appearance of the stool, a careful examination of at least 4–6 smears should be made before pronouncing it negative. If anything suspicious is seen, a very careful examination should be made, making as many smears as may be necessary. If no decision can be reached, repeated stool examinations should be made by direct examination, by culture, and iron-hematoxylin preparations if necessary.

VI. Microscopic Examination

Things necessary for efficient work:

A good microscope

Objectives { Low—2/3″ / High dry—1/6″ / Oil immersion—1/12″ }

Oculars—10 is indispensable (4 or 6 may also be of need)

Ocular micrometer—properly calibrated

Good light; artificial is best because it is always the same.

EXAMINATION

Bloody mucous stools from active cases of amebic dysentery—Specimens may be obtained with the proctoscope on proctoscopic examination, by means of a rectal tube, by inserting a glycerin suppository, or normally. A tiny granule of bloody mucus is chosen, placed on a slide, covered with a cover slip and examined under low power first and then under high dry for active *E. histolytica* with red blood cells in them. If the stool should contain fecal material it should be diluted first with a little warm saline to the proper consistency. This is done by placing a drop of warm saline on a slide, a little of the stool is taken on an applicator or needle and emulsified (mixed well) to reading opacity in the drop of saline, covered with a cover slip, and examined as above. The diaphragm of the microscope should be closed as much as possible.

VII. Examination of Stools from Convalescent Cases and Carriers

As the diarrhea of the active cases of amebic dysentery subsides following medication, the feces remains longer in the large bowel, the active amebae lose their motility and begin to round off, giving rise to precysts. The precysts of *E. histolytica* are 20–30 μ in diameter, hyaline in appearance, and are free from granular material, or contain only very fine granular material (the remains of the red blood cells). The nucleus is typical, containing a small central karyosome, and is bounded by a fine membrane composed of fine chromatin granules. In this stage it is sometimes difficult to diagnose *E. histolytica*. Such stools may be cultured (on liver infusion, agar overlaid with serum, saline, described later), whereupon the precysts vegetate, giving rise to typical *E. histolytica* trophozoites. If difficulty is still encountered, the examinations should be repeated on normal stools as well as those following salts. Iron-hematoxylin preparations might also be made and examined for typical *E. histolytica* with the oil immersion lens.

As the stools remain longer in the large bowel they become more formed and the amebae encyst, giving rise to cysts containing 1–4 nuclei.

Formed stools from convalescent cases or from carriers should be examined with warm water or saline as well as with iodine. (The iodine solution should be fresh—a 1 per cent aqueous solution of iodine in 2 per

cent potassium iodide, or an aqueous solution of 5 per cent potassium iodide saturated with iodine and diluted with an equal part of distilled water for use. This should be kept in a brown bottle and away from the light, or a card may be wrapped around the bottle to keep the iodine solution from disintegrating.)

In making smears a drop of saline or water is placed on a slide. In this is emulsified a small amount of feces, with a needle or applicator, to reading opacity, covered with a cover slip, and examined first with the low power and then with the high dry (some people prefer to use the oil immersion lens). In such smears may be seen flagellates, amebae, ova, larvae of such worms as *Strongyloides*, etc. In young cysts the chromatoid bodies show up very clearly in the water or saline preparations. Several smears of each specimen should be examined with saline or water, as well as with iodine. In the iodine preparations iodine instead of water or saline is used and smears made and examined exactly the same as above. The iodine kills the protoplasm and stains the cytoplasm more or less yellow, the cyst and nuclear walls dark, and the glycogen dark brown. As a rule, the karyosomes stand out as refractile bodies. Thus one can study the number and type of nuclei, thickness of the cyst wall, type and position of the karyosome, etc. Iron-hematoxylin permanent preparations are unnecessary unless finer details are desired to study.

VIII. Examination of Pus from Liver and Lung Abscesses

In examining pus from liver and lung abscesses one should look for active *E. histolytica*, for they do not encyst in pus. *E. histolytica* are found in the walls of abscesses and not in the central necrotic material, so that care should be taken to procure material from the walls of such abscesses in examining for *E. histolytica*.

In case of doubt as to the diagnosis of an intestinal protozoan, or if permanent preparations are desired for other reasons, the following method has been found to work the best: The material should be perfectly fresh, properly fixed, and at no time in the process should the slide or cover slip preparations be permitted to dry; as soon as that happens the preparations are ruined and should be discarded. Bloody mucous or soft stools need not be diluted, but hard stools should be diluted with saline before making smears. Smears may be made on slides or cover slips, using an applicator. Smears should be uniform and not too thick. If slides are used, the fixing fluid should be placed in staining jars. If cover slips are used, the fixing fluid is placed in Petri or evaporating dishes. Smears of the proper thickness are made and the cover slips are immediately dropped film side downward into the fixing fluid and are permitted to float for a few moments, then they are picked up with forceps and completely immersed face upward, using plenty of fixative and discarding it when through.

Procedure for Fixing and Staining (Iron-hematoxylin Method)

Fixing solution—Schaudinn's solution

Saturated solution of mercuric chloride in distilled water.. 2 parts
95% alcohol . . . . . 1 part

To every 100 c.c. of this mixture add 2-5 c.c. glacial acetic acid

Fix 10–20 minutes (30 minutes is better for cysts)

Wash and harden

50% alcohol for a few minutes

To remove fixation, 70% alcohol to which a few drops of iodine solution (alcoholic) has been added, for at least 10 minutes. The iodine removes the remaining $HgCl_2$.

95% alcohol (best for an hour or several days)

Hydrate

70% alcohol for a few minutes

50% alcohol for a few minutes
30% alcohol for a few minutes
Distilled water
Stain (Iron-hematoxylin method)
Mordant by placing in 2% aqueous iron alum (ammonium ferric sulphate) 2–6 hours
Wash in distilled water for a few seconds
Stain in 0.5% ripened solution of hematoxylin in distilled water 6–24 hours. (Make up solution, place in flask, plug with cotton wool, place in a warm place—in the sun, if possible—shaking from time to time. When the solution is "ripe"—a good brown color, namely the hematoxylin is more or less oxidized to hematein, it is ready for use. This may require several weeks.)
Wash in distilled water
Differentiate in 0.5 to 1% aqueous iron alum (never permitting the preparation to dry during the process). Remove a slide, rinse in distilled water, and examine under the microscope for proper differentiation; the cytoplasm should be gray; the cyst, nuclear walls, as well as the chromatoid bodies and karyosomes, should be black when properly decolorized.
Wash in distilled water
Then in running tap water for at least ½ hour in order to wash out all the alum; otherwise the decolorization will continue
Wash in distilled water
Dehydrate
50% alcohol 5 minutes
70% alcohol 5 minutes
95% alcohol 5 minutes
Absolute alcohol 10 minutes, 2 changes (Absolute alcohol and xylol, 50–50, 5 to 10 minutes)
Clear (Xylol 5 minutes)
Mount in balsam.

IX. Concentrating Stools for Cysts

A number of methods may be used in concentrating specimens for cysts when these are present in small numbers. One method is to take a small piece of the stool, emulsify it well in water or saline, strain through two layers of gauze, centrifuge at moderate speed 2 or 3 minutes, pour off the supernatant fluid, emulsify the sediment as before, and repeat the washing 2 or 3 times. Examine the final sediment with water and iodine.

Another method is to emulsify a small piece of feces with distilled water, place it in a 500 c.c. graduate, make up to 500 c.c. with distilled water, mixing well. Let it stand for 20 to 30 minutes, pour off the supernatant fluid and replace with distilled water, mix well and let stand over night. Then examine the sediment with water and iodine solution.

X. Cultural Methods

At times cultures are of great assistance in making a diagnosis because precysts, cysts, and atypical trophozoites become typical trophozoites showing the characteristic morphology and motility. However, the cultures must be made properly and carefully, using proper media, aseptic methods, and examining at the proper time. Cultures should not be relied upon entirely because our experience during the Chicago epidemic has taught us that only about 5–8 per cent of the small variety *E. histolytica* grow in the culture medium used for the large variety. The presence of arsenic and other amebicides prevent *E. histolytica* from growing. Routine cultures are unnecessary in making a survey.

MEDIA

Cleveland and Collier (Liver infusion agar)

This medium is one of the best for practical diagnostic purposes. The medium consists of slants of liver infusion agar.

30 gm. liver infusion agar (Digestive Ferments Co., Detroit, Mich.)
3 gm. dehydrated sodium phosphate
1,000 c.c. distilled water
Dissolve, tube about 5 c.c. per tube, sterilize 15 lb. for 30 minutes, slant and keep in the ice box until shortly before using.

Before making inoculations these slants are overlaid with sterile serum saline 1–6. (Wassermann negative hu-

man inactivated serum, horse or rabbit serum, etc., may be used by taking 1 part of serum to 6 parts of NaCl, filtering through a sterile Berkefeld filter, incubating 24–48 hours to test sterility. If sterile, place in the ice box until shortly before using.)

The slants and serum saline should be placed in the incubator for a couple of hours before using in order to bring the temperature up to body temperature. Sterile precautions should always be used in making the cultures. Before making inoculations the slants are overlaid (sterilly) with sterile serum NaCl, 1–6. A little sterile rice starch or rice flour may be added, but is not necessary. If the stool is soft, watery, or bloody mucus, a little is inoculated, using a sterile pipette or needle. If the stool is solid, a loopful is inoculated, macerating well by rubbing with the needle against the sides of the tube. Incubate at 37.5° C., and examine after 24 and 48 hours. Cultures should not be examined prematurely because occasionally *E. coli* grows in this medium, and in young cultures when rapid division takes place the nuclear structure may not be typical, the motility rapid; so that one may have difficulty in differentiating *E. histolytica* from *E. coli*. Occasionally sub-cultures are necessary in order to decide as to whether an organism is *E. histolytica* or *E. coli*. In 24–48 hour cultures, even when *E. coli* is present, it shows its typical characteristics—thick wall, sluggish motility, the entire organism seems to roll about in its wall. *E. histolytica*, on the other hand, has a thin wall and moves rapidly by means of a pseudopodium in an elongating, slug-like manner.

"Mature living *E. histolytica* cysts do not all hatch simultaneously nor at the same rate; some hatch quickly, others slowly. They usually begin to hatch after 2¼ hours' incubation. Cysts which do not hatch after 12 hours' incubation usually perish. Occasionally some hatch after 20 hours' incubation. Cysts begin to hatch when the chromatoid bodies and glycogen disappear." C. Dobell, *Parasit.* XX, 4:383, 1928.

All other intestinal amebae do not grow as readily in this culture medium and cannot be maintained in culture as long as *E. histolytica*. When the other intestinal amebae grow in this culture medium they show their typical characteristics and should not be confused with *E. histolytica*.

In order to keep strains of *E. histolytica* going, make sub-cultures every 48 hours (using sterile precautions). A little sterile rice flour or rice starch is added to the liver infusion agar slant overlaid with serum saline before making the inoculations. To make the sub-cultures, use a sterile pipette with a good sized bore and transfer a little of the sediment, taking as little of the supernatant fluid as possible. Some strains of *E. histolytica* grow readily, while others grow with great difficulty.

Locke Serum Medium of Craig

This medium consists of a mixture of a modified Locke solution and inactivated human, horse or rabbit serum. The serum used should not be over 48 hours old.

Locke solution:

Sodium chloride	9.0	gm.
Calcium chloride	0.024	gm.
Potassium chloride . .	0.42	gm.
Sodium bicarbonate. .	0.20	gm.
Distilled water	1,000	c.c.

Filter and autoclave 15 lb. 15 minutes

Medium:

1 part inactivated (56° C. ½ hour) serum to 7 parts Locke solution
Filter through a Berkefeld filter
Tube 10 c.c. per sterile tube
Incubate 24–36 hours to test sterility
Keep in incubator until used.

A slight amount of sterile rice starch or rice flour increases the efficiency of this medium.

XI. COMPLEMENT-FIXATION IN THE DIAGNOSIS OF AMEBIASIS AND AMEBIC DYSENTERY

The technic is practically the same as that used in the standard method for the complement-fixation of syphilis in the United States Army laboratory fully described in Craig's "The Wassermann Test" (1920), except that the specific amebic antigen is used. The anti-human as well as the anti-sheep system may be used.

Reagents needed:

The blood serum to be tested is inactivated in a water-bath at 56° C. for ½ hour.

Antigen—alcoholic extract of sediment of cultures of *E. histolytica*.

Antigen Control—prepared exactly the same way as the test antigen except that the bacteria growing in association with the *E. histolytica* are used.

Amboceptor—anti-human or anti-sheep, as the case may be.

Complement Guinea Pig Serum:

Red blood cells—type 4 (Moss) human or sheep cells (whatever system is used).

Set up of test:

Front and rear tubes
Place 0.9 c.c. NaCl in each tube
Test 0.1 c.c. unknown serum in front and rear tubes
0.1 c.c. + serum in front and rear tubes
0.1 c.c. — serum in front and rear tubes
1 unit antigen to front tubes
2 units complement to each tube
Incubate—water-bath 37.5° C. ¼ hour
Add 0.5 c.c. 3% cells (Craig uses 0.1 c.c. of 5%)
Add 2 units amboceptor
Incubate 37.5° C. water-bath for 1 hour
Shake every 15 minutes
Place in ice box for 2 hours and read.
Readings are made as follows: 4+, 3+, 2+, + and ±, according to the degree of inhibition of hemolysis.

Any reaction below 3+ should not be considered diagnostic unless the patient has been treated. Upon treatment the complement-fixation becomes negative.

The complement-fixation test is still in the experimental stage and one may use the test only as an aid and not as an absolute criterion in making a diagnosis. As little as one injection of emetine hydrochloride will change a positive complement-fixation reaction to negative when the stools may still show motile amebae.

BERTHA KAPLAN SPECTOR,
Referee

Tentative Methods for the Serological and Bacteriological Procedures in the Diagnosis of Enteric Fevers*

Previous Report and Special Problems
Serological Procedures
I. Outfits
II. Preparation of specimen
III. Dilutions
IV. Agglutinating suspensions Preparation
V. The test
VI. Reactions
VII. Reports
Bacteriological Procedures
Examination of Blood
I. The outfit
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I. The outfit
II. Plating
A. Feces
B. Urine
Identification of Species
Standardization of Antisera
Standardization of Media
Preparation of Media and Reagents
References

The report submitted to your committee in 1934 outlined the fundamental procedures recommended as aids in the diagnosis of enteric disease. These were based upon reports in the literature, information obtained by means of questionnaires sent to directors of laboratories representative of various sections in the United States and Canada, and personal experience. The outline was published in the *Year Book* of the Association in the hope that persons other than those who had received the questionnaire would submit comments and criticisms. While the response has been limited, the data now available would seem sufficient to warrant the inclusion of details of technic in the outline previously prepared.

Appended to the 1934 report was a list of problems which seem particularly vital to the development of the most efficient and reliable methods employed as aids in the diagnosis of enteric disease. Considerable interest has been shown by certain investigators, and in some instances contributions have been made. However, as would be expected, there was lack of uniformity in the studies undertaken by the various workers and in the type of data furnished. It is suggested, therefore, that a selected group of laboratories be invited to participate in a coöperative investigation of at least certain of the problems, in which a carefully planned mode of procedure would be followed.

The problems previously suggested for coöperative study are listed for reference.

Problems for Coöperative Study

Serological Procedures

I. Comparison of various types of suspension for demonstrating the granular type of agglutination with *B. typhosus*.

II. Study of the diagnostic significance of macroscopic agglutination tests with suspensions of: (1) *B. paratyphosus* A, (2) *B. paratyphosus* B, (3) *B. dysenteriae*, and (4) other mem-

* Report of the Referee on Methods and Reagents for the Diagnosis of Enteric Fever for the Standard Methods Committee on Diagnostic Procedures and Reagents.

bers of the enteric disease group in patients' sera.

III. Selection of standard strains for the tests mentioned in I and II.

IV. Study of other serological tests, for example, complement-fixation and precipitation tests, in the diagnosis of enteric disease.

BACTERIOLOGICAL PROCEDURES

I. Selection of the optimum preservative for fecal and urinary specimens when the bacteriological examination for microörganisms of enteric disease cannot be undertaken immediately after collection.

II. Determination of the etiological significance of species concerning which there is, at present, difference of opinion, for example, *B. morgani* No. 1, *B. alkalescens*, *B. dispar*, etc.

III. Establishment of criteria for the most rapid and accurate identification of species, particularly those belonging to the dysentery and paratyphoid-enteritidis group.

SEROLOGICAL PROCEDURES

EXAMINATION OF SERUM FOR AGGLUTINATION WITH B. TYPHOSUS

I. *The outfit*—Use a sterile, tightly stoppered or sealed, glass container with a capacity of from 10 to 15 c.c., in a mailing case conforming to the postal laws and regulations.

II. *Preparation of the specimen*—Remove the serum with a sterile pipette (a capillary pipette fitted with a small rubber bulb may be used) with precautions to prevent contamination of the clot, which should be reserved for bacteriological examination. Centrifugalize to remove the blood cells.

Results obtained with hemolyzed sera should be considered of questionable significance, since certain conditions which produce hemolysis may also affect the agglutinative properties. The clots from such specimens should be cultured. The hemolysis may be due to the development of the bacteria which were present in the blood stream.

III. *Dilutions*—Prepare the following dilutions of the serum in 0.85 per cent solution: 1:10, 1:20, 1:40, 1:80, 1:160, and higher if desired.

IV. *Agglutinating suspensions*—Employ at least two suspensions of uniform turbidity; one to demonstrate floccular and the other, granular agglutinative properties.

Preparation of Formalin-Treated Suspension to Demonstrate Floccular Agglutination — Employ a "smooth," actively motile strain of *B. typhosus*. Streak a freshly poured plate of beef-infusion agar containing no water of condensation. If, after from 18 to 20 hours' incubation at from 35 to 37° C., other than perfectly "smooth" colonies are noted, suspend two or three of the latter in small amounts of salt solution or broth and replate immediately. Repeat this process until an entirely "smooth" culture is obtained. Check the purity of the culture by microscopic examination, by determining the reaction in double-sugar medium and agglutination in *B. typhosus* anti-serum. Use a suspension of a 24 hour beef-infusion agar culture to inoculate 2 per cent beef-infusion agar in pint Blake or other bottles which will give adequate surface. If water of condensation is present on the agar, remove it with a pipette before inoculation. After from 18 to 20 hours' incubation at from 35 to 37° C., pipette into each pint Blake bottle or its equivalent 10 c.c. of 0.85 per cent salt solution containing 2 per cent of formalin and suspend the growth in this. Make Gram-stained preparations and discard suspensions which show the presence of contaminating microorganisms. Pool the suspensions in a sterile bottle; place in the refrigerator; and after 48 hours, prepare sub-cultures. Hitchens's medium is recommended for this purpose since it provides conditions favorable to both aerobic and anaerobic microörganisms.

Should growth of *B. typhosus* occur, retest, after additional refrigeration, until no evidence of viability is obtained. If considerable material settles out, decant the supernatant suspension into a fresh bottle. Adjust the suspension to a turbidity 10 times that of barium-sulphate * standard No. 3 [1] and a formalin content of 2 per cent. Determine the opacity of the suspension by comparison of diluted portions with barium-sulphate standard No. 3 and with a similar suspension previously standardized. Calculate the required amount of diluents as in the following example:

Assuming that 400 c.c. of concentrated suspension are to be adjusted and it is found that this needs to be diluted 1:12 to equal barium-sulphate standard No. 3, then:

$$\frac{400 \times 12}{10} = 480 \text{ c.c.—volume adjusted suspension}$$

or 80 c.c. of salt solution containing 2 per cent formalin are required to be added to 400 c.c. of the original suspension.

For agglutination tests, dilute with 9 parts of 0.85 per cent salt solution, thus obtaining a suspension equivalent in turbidity to barium-sulphate standard No. 3 and containing 0.2 per cent formalin.

Test the agglutinability of the suspension in comparison with a previous lot which has proved satisfactory, using, if possible, a series of human sera giving various degrees of agglutination as well as antisera produced in animals. Store in a refrigerator.

Preparation of Alcohol Treated Suspension [2] *to Demonstrate Granular Agglutination*—Employ a "smooth" strain of *B. typhosus*, preferably one which is nonflagellated. Follow the procedure outlined for the formalin treated suspension to insure smoothness and purity of the culture and inoculate the agar in pint Blake or other bottles. After from 18 to 20 hours' incubation, pipette into each pint Blake bottle or its equivalent 10 c.c. of 0.85 per cent salt solution containing 0.5 per cent phenol and suspend the growth. Make Gram-stained preparations and discard suspensions which show the presence of contaminating microörganisms. Measure the suspension in a sterile graduate and pool it in an Erlenmeyer flask. Add alcohol to give a concentration of 33.3 per cent. The volume of 95 per cent alcohol required equals the volume of the suspension multiplied by 0.54, a constant obtained from the formula:

$95\ x = 33.3\%$ (volume of the suspension + x) in which x = the volume of 95 per cent alcohol which will be required

Rotate the flask slowly as the alcohol is added or stir with a sterile glass rod. Pour the alcohol treated suspension into sterile cylinders and incubate over night at from 35 to 37° C. The following morning, decant the supernatant suspension from the sediment into a sterile bottle, mix thoroughly, and prepare sub-cultures to determine the presence of contaminating microorganisms. Hitchens's medium is recommended for this purpose since it provides both aerobic and anaerobic conditions.

Adjust the turbidity of the suspension to 10 times that of barium-sulphate standard No. 3 and its alcohol content to 30 per cent. Determine the opacity of the suspension by comparison of diluted portions with barium-sulphate standard No. 3 and with a similar suspension previously standardized. Calculate the required volume of diluents as in the following example:

Assuming that 400 c.c. of suspension are to be adjusted and that this needs

* Silica or other standards of equivalent opacity may be employed.

to be diluted 1:12 to obtain the required density, then:

$\frac{400 \times 12}{10} = 480$ c.c.—volume of adjusted suspension and 80 c.c.—total volume of diluent required

$480 \times 30\% = 144$ c.c.—total volume of 100 per cent alcohol required

$400 \times 33.3\% = 133.3$ c.c.—volume of alcohol already in suspension

$144 - 133.3 = 10.7$ c.c.—volume 100 per cent alcohol to be added

$10.7 \times \frac{100}{95} = 11.3$ c.c.—volume of 95 per cent alcohol required

80 c.c. $- 11.3$ c.c. $= 68.7$ c.c.—volume of 0.85 per cent salt solution containing 0.5 per cent phenol to be added.

For agglutination tests, dilute with 9 parts of 0.85 per cent salt solution, thus obtaining a suspension equivalent in turbidity to barium-sulphate standard No. 3 and containing 3 per cent alcohol and 0.05 per cent phenol.

Test the agglutinability of the suspension in comparison with a previous lot which has proved satisfactory, using, if possible, a series of human sera giving various degrees of agglutination as well as antisera produced in animals. Store in a refrigerator.

V. *The test*—Combine equal parts (from 0.3 to 0.5 c.c. recommended) of serum dilutions and suspensions in small, clear glass tubes (11 mm. x 75 mm. is a convenient size), and shake thoroughly. For purposes of control, combine equal parts of each suspension and (a) 0.85 per cent salt solution and (b) specific dilutions of homologous antiserum. Incubate for from 18 to 20 hours at approximately 50° C.*

VI. *The reaction*—Record the degree of reaction as follows:

4+ = clear supernatant fluid—complete agglutination

3+ = clear supernatant fluid—definite agglutination

2+ = cloudy or slightly cloudy supernatant fluid—definite agglutination

+ = cloudy supernatant fluid—agglutination discernible to the unaided eye

± = questionable reaction

— = uniformly turbid suspension—no agglutination

VII. *The report*—Either of the following methods may be employed.

First method

A. Report that definite agglutination occurred—

1. When both granular and floccular agglutination (3+ or 4+) is obtained in a 1:80, or higher, dilution.

2. When either granular or floccular agglutination (3+ or 4+) is obtained in a 1:160, or higher, dilution.

B. Report that partial agglutination occurred—

1. When reactions less than those reported as definite agglutination are obtained, provided agglutination (3+ or 4+) of one or both types occurs in a 1:40 dilution.

C. Report that agglutination of diagnostic significance did not occur—

1. When reactions less than those regarded as partial are obtained.

2. When no agglutination is obtained.

or

Second method

A. Report the dilution of serum in which each type of agglutination is obtained.

In case either granular or floccular agglutination alone occurs, add one of the following statements to the report or write a letter to the physician relative to the significance of the reaction.

" Floccular but not granular agglutination occurred. This type of reaction is occasionally obtained in sera from patients having typhoid fever but is more often observed in blood from typhoid carriers or those who have re-

* This should not vary more than 2° below or above 50° C.

ceived typhoid vaccine. If the individual is ill or there is evidence that he may be a typhoid carrier, fecal specimens should be submitted for bacteriological examination."

or

"Granular but not floccular agglutination occurred. This type of reaction is observed in sera from a small percentage of patients having typhoid fever but may be obtained when there is an infection with a microörganism antigenically related to *B. typhosus*. Therefore, fecal specimens should be submitted for bacteriological examination."

EXAMINATION OF SERUM FOR AGGLUTINATION WITH B. PARATYPHOSUS A AND B

The procedure outlined for *B. typhosus* may be followed. However, the relatively low incidence of infections with microörganisms of the paratyphoid-enteritidis group in this country and the variety of species involved render the agglutination test of questionable practical value for use as a routine procedure.

BACTERIOLOGICAL PROCEDURES FOR THE ISOLATION OF BACILLI INCITING ENTERIC DISEASE

EXAMINATION OF BLOOD

I. *The outfit*—Use a sterile, tightly stoppered or sealed, glass container with a capacity of from 10 to 15 c.c., in a mailing case conforming to the postal rules and regulations.

II. *Preparation of the specimen*—Remove the serum with a sterile pipette, using precautions to prevent contamination of the clot. Reserve the serum for serological tests.

III. *Cultural examination*—Transfer the clot to 8 or 10 c.c. of bile medium (Conradi's modified). Sub-culture after from 40 to 48 hours' incubation at from 35 to 37° C. and at intervals thereafter for at least 3 weeks, unless bacterial incitants of enteric disease are isolated sooner.

EXAMINATION OF FECES AND URINE

I. *The outfit*

A. Use a tightly stoppered or sealed, glass tube or jar, with a capacity of from 20 to 30 c.c. in a mailing case conforming to the postal laws and regulations. The tube or jar should contain from 8 to 10 c.c. of 30 per cent glycerol in 0.6 per cent salt solution.

B. Give instructions for the collection of the specimens, printed either on the history form or on a separate slip included in the outfit.

C. Provide a swab or wooden spoon, for the collection of feces, of which about 1 gm. should be added to from 8 to 10 c.c. of glycerol solution.

D. From 8 to 10 c.c. of urine should be added to from 8 to 10 c.c. of glycerol solution.

NOTE: When specimens of feces or urine can be examined within a very short time (1 or 2 hours) after collection, and when urine has been collected aseptically, the use of a preservative is, of course, unnecessary.

II. *Plating*

A. *Feces*—If undiluted feces is submitted, combine 1 part with from 8 to 10 parts of fluid medium, preferably 30 per cent glycerol solution, and allow it to stand for from 10 to 15 minutes so that the coarse particles will settle. Use not less than 4 plates, at least 1 of which shall contain medium favorable to the development of dysentery bacilli (eosin-methylene-blue agar or a modification of Endo's agar) and another, that which is inhibitory to *B. coli* but favorable to typhoid and paratyphoid bacilli (Krumwiede's brilliant-green agar). Usually, the amount of fecal suspension which can be transferred with a loop 4 mm. in diameter on each plate will give well isolated colonies. Streak the material on each plate with a large wire loop, bent wire needle or sterile glass rod. Incubate the plates for from 18 to 20 hours at from 35 to 37° C.

B. *Urine*—Streak the specimen as received on one plate of eosin-methylene-blue or Endo's agar and one of brilliant-green agar. Add from 2 to 3 c.c. of the remainder to from 8 to 10 c.c. of bile medium (Conradi's modified). Incubate the latter for from 18 to 20 hours at from 35 to 37° C. and streak one plate of eosin-methylene-blue or Endo's agar and one of brilliant-green agar.

For purposes of control, streak portions of each type of plating medium with cultures of *B. typhosus, B. paratyphosus* A and B, *B. dysenteriae,* and *B. coli.*

CRITERIA FOR IDENTIFICATION OF SPECIES

The criteria for identification of bacterial incitants of enteric disease and other Gram-negative bacilli found in feces have, for convenience, been summarized in Table I. The procedures necessary to determine the significant characteristics will be discussed separately.

I. *Colonial characteristics*—These should be considered only as a means for selecting those colonies from which sub-cultures are to be made. If a tentative report seems essential, a suspension of microörganisms from a colony may be tested for agglutination in a loopful of a dilution of antiserum on a glass slide, but a final report should be based on adequate criteria for identification of the species.

II. *Differential media for sub-cultures*—Pick characteristic colonies to Russell's double-sugar medium. Stab the butt and streak the surface of the slant. Record the reactions after incubation for from 18 to 20 hours at from 35 to 37° C. (For purposes of control, inoculate tubes of this medium with standard strains of *B. typhosus, B. paratyphosus* A and B, *B. dysenteriae,* and *B. coli.*)

III. *Morphology*—Prepare films on glass slides or, if slide agglutination tests are performed, allow these preparations to dry and then fix them and stain by Gram's method. All the microörganisms listed in Table I are relatively small, evenly staining, Gram-negative bacilli without spores. If the stained preparations show any evidence of contamination, streak the culture on blood agar as well as Endo's agar since the contaminating microörganism may be inhibited on the latter. Prepare a hanging drop to study the motility. Transfer one loopful of a 1 to 3 hour broth culture to a cover slip and invert over it a hollow-ground slide which has been ringed with vaseline. Examine with a high-power dry objective.

IV. *Macroscopic slide agglutination test*—Transfer to a glass slide one loopful of appropriate antiserum diluted as indicated by previous standardization and a loopful of a corresponding dilution of serum from a normal animal of the species used for the production of the antiserum. Transfer, with a small loop, growth from a colony on the plating medium or from Russell's double-sugar agar to the normal serum and after emulsifying, transfer a loopful to the antiserum. Tilt the slide slowly and observe over a black background for clumping.

A report should not be based on the results of this test unless the species indicated has been isolated from a previous specimen from the same patient. The test may be very useful, however, in the selection of antisera in which to perform macroscopic tube agglutination tests.

V. *Macroscopic tube agglutination test*—Inoculate beef-extract broth from the double-sugar slant and incubate at from 35 to 37° C. (usually from 2 to 4 hours) until the turbidity is equivalent to that of barium-sulphate standard No. 3, or suspend the growth from a beef-extract or beef-infusion agar

TABLE I

CRITERIA FOR IDENTIFICATION OF BACTERIA INCITING ENTERIC DISEASE AND OTHER GRAM-NEGATIVE BACILLI FOUND IN FECES*

Species	Reaction in double-sugar medium	Reaction in media containing: Dextrose	Maltose	Mannitol	Lactose	Saccharose	Xylose	Rhamnose	Dulcitol	Motility	Indol production	Reaction in litmus milk	Serological reaction
B. typhosus	+†	A	A	A	—	—	$\overline{A}$	—	($\overline{A}$)	Motile[1]	Not produced	A ≏ – or Alk ≏	Agglutination in *B. typhosus* antiserum
B. dysenteriae Shiga	+	A	$\overline{A}$	—	—	—	—	—	—	Nonmotile	Not produced	A ≏ – or Alk ≏	Agglutination in polyvalent and/or monovalent antisera for members of the group
Schmitz	+	A	$\overline{A}$	—	—	$\overline{A}$	—	A	—	Nonmotile	Produced	A ≏ – or Alk ≏	
Flexner°	+	A	$\overline{A}$	A	—	$\overline{A}$	—	($\overline{A}$)	($\overline{A}$)	Nonmotile	Variable	A ≏ – or Alk ≏	
Sonne	+	A	A	A	–A	–A	—	A	—	Nonmotile	Not produced	A or AC	
B. dispar	+	A	A	A	–A	$\overline{A}$	$\overline{A}$	$\overline{A}$	$\overline{A}$	Nonmotile	Produced	A or AC	Of questionable value
B. alkalescens	+	A	A	A	—	—	A	A	A	Nonmotile	Produced	Alk or A to Alk	Agglutination in *B. alkalescens* antiserum
B. paratyphosus A	⊕	AG	AG	AG	—	—	—	AG	AG	Motile[1]	Not produced	A or Alk	Agglutination in, and absorption of, agglutinative properties from *B. paratyphosus* A antiserum
Paratyphoid-enteritidis group‖	⊕	AG	AG	AG	—	—	AG (—)	AG	AG (—)	Motile[1]	Not produced	A to Alk	Agglutination in polyvalent and/or monovalent antisera for members of the group. Absorption of agglutinative properties is necessary for the identification of species.
B. morgani No. 1	⊕	AG	—	—	—	$\overline{AG}$	—	—	—	Motile[1]	Produced	– or Alk	Of questionable value
B. coli	⊕	AG	AG	AG	AG	$\overline{AG}$	AG	AG	AG	Motile[1]	Produced	AC	Of questionable value

* Only those species which do not liquefy gelatin are included.
Variations, especially in fermentative properties, which have not been indicated may be occasionally encountered, particularly after long cultivation on artificial media.

† Occasionally acid throughout.

[1] Nonmotile strains sometimes found, especially when freshly isolated.

° The mannitol-fermenting group.

‖ *B. paratyphosus* B, *B. enteritidis* and other closely related species.

\+ = Acid butt, neutral or alkaline slant, no gas.

⊕ = Acid and gas in butt, neutral or alkaline slant.

⊕ = Acid and gas throughout.

A = Acid.

– = No perceptible change. –A = Acid produced slowly. AG = Acid and gas.

$\overline{A}$ = No change or acid. ($\overline{A}$) = Usually no change, occasionally acid.

$\overline{AG}$ = No change or acid and gas.

(—) = Usually acid and gas, occasionally no change.

A ≏ = Weak acid.

Alk = Alkaline.

Alk ≏ = Weak alkaline.

AC = Acid and coagulation.

slant which has been incubated for from 18 to 20 hours, in 0.85 per cent salt solution making the turbidity equivalent to that of barium-sulphate standard No. 3. Combine 0.3 c.c. amounts of either of these and of appropriate antisera diluted as indicated by previous standardization. For purposes of control, combine 0.3 c.c. amounts of each culture to be tested with salt solution and of dilutions of each antiserum with a suspension of its homologous species. Incubate the tests at from 35 to 37° C. for 2 hours and then leave in the refrigerator over night before reading, except in the case of *B. alkalescens* and *B. dysenteriae* Sonne. Incubate tests for identification of these two species at approximately 50° C. for from 18 to 20 hours and read. Follow the procedure outlined under serological procedures for recording the degree of reaction.

VI. *Fermentative properties*—Inoculate carbohydrate peptone water containing Andrade indicator and observe at frequent intervals during 14 days' incubation at from 35 to 37° C. Test this medium each time a new lot is prepared with standard strains of the species included in Table I. When there is any doubt concerning the production of gas, inoculate dextrose broth in a Durham fermentation tube and observe at intervals during 1 week's incubation at from 35 to 37° C. Incubate one tube of the same lot of medium inoculated with *B. coli* and one uninoculated and observe at the same time as the culture being studied. When gas production is to be determined from carbohydrates which are easily hydrolyzed by heat, use medium sterilized by filtration distributed in Smith fermentation tubes.

VII. *Production of indol*—Inoculate a tube of Dunham's peptone water with the culture to be tested and incubate for 4 days at from 35 to 37° C. Incubate at the same time one tube of this medium inoculated with *B. coli*, one with *B. typhosus*, and one uninoculated. Add 1 c.c. of ether to each and mix well by rotating the tubes. Allow a few drops of Ehrlich's reagent to run down the side of the tube to form a layer between the ether and the peptone water. A definite ring varying from rosy pink to deep red indicates the presence of indol.

VIII. *Liquefaction of gelatin*—Transfer the culture with a needle, stabbing the medium to the bottom of the tube. Incubate at from 35 to 37° C. for 2 weeks unless evidence of liquefaction is observed sooner. To determine liquefaction, immerse the tube in ice water or leave it in the refrigerator for 30 minutes.

IX. *Reaction in litmus milk*—Inoculate a tube of litmus milk with the culture to be tested and record the reaction at frequent intervals during incubation at from 35 to 37° C. for 14 days. For purposes of comparison, incubate at the same time an uninoculated tube of the same lot of litmus milk.

X. *Absorption tests*—Occasionally, when the results of agglutination tests are indefinite or when cross-agglutination is obtained, it is necessary to employ absorption tests to determine the species.

Combine equal parts of a dilution of antiserum and (a) a suspension of the homologous species and (b) a suspension of the unidentified culture grown for from 18 to 20 hours at from 35 to 37° C. on beef-infusion agar. The serum dilution and the turbidity of the absorbing suspensions are determined by standardization. Incubate the mixtures for 3 hours at approximately 50° C., inverting the tubes occasionally to insure contact of serum and microörganisms. Leave them in the refrigerator over night and centrifugalize at high speed for 1 hour

or until the supernatant fluid is clear. Prepare dilutions of the supernatant fluid, and combine 0.3 c.c. of each with 0.3 c.c. of a suspension in 0.85 per cent salt solution (comparable in turbidity to barium-sulphate standard No. 3) of (a) the species homologous to the serum used and (b) the culture to be identified. Incubate the tests at from 35 to 37° C. for 2 hours and leave them in the refrigerator over night before reading the reactions. Incubate tests with absorbed *B. alkalescens* and *B. dysenteriae* Sonne antisera at approximately 50° C. for from 18 to 20 hours and read.

For control purposes, perform tests, at the same time, with sera which have not been subjected to absorption.

It may be unnecessary to study all of the characteristics listed in Table I, but when a strain differs from the standard in any property it should be studied most carefully. This should be done also before reporting members of the paratyphoid-enteritidis group since strains of *B. coli* and of the so-called paracolon group may be agglutinated in sera prepared with certain of the pathogenic species. The similarity in biochemical and other properties and the close serological relationship of members of the paratyphoid-enteritidis group render the identification of individual species an arduous procedure. Furthermore, this is of little practical value because of the relatively low incidence in this country of infections with members of this group. The diagnostic significance of *B. alkalescens, B. dispar,* and *B. morgani* No. 1 has not been established but they are encountered with sufficient frequency to warrant recognition.

STANDARDIZATION OF ANTISERA

I. *Macroscopic slide agglutination tests*—Dilute the antiserum 1:25, 1:50, 1:100, 1:200, and higher if necessary, with 0.85 per cent salt solution. Perform macroscopic slide agglutination tests with a standard strain of the homologous species and with the other species of the bacilli inciting enteric disease. Test a similar series of dilutions of serum from a normal animal of the same species as that in which the antiserum was produced. If agglutination is obtained in the latter, procure a satisfactory strain of the species and repeat the tests. Select for use in routine tests the lowest dilution of antiserum in which definite agglutination occurs with the homologous but none with the heterologous species.

II. *Macroscopic tube agglutination tests*—Dilute the antiserum 1:100, 1:250, 1:500, 1:1,000, 1:2,000, and higher if necessary, with 0.85 per cent salt solution. Perform macroscopic tube agglutination tests with a standard strain of the homologous species and with the other species of the bacilli inciting enteric disease. Test a similar series of dilutions of serum from a normal animal of the same species as that in which the antiserum was produced. If agglutination is obtained in the latter, procure a satisfactory strain of the species and repeat the tests. Select for use in routine tests the two lowest dilutions of antiserum in which definite agglutination occurs with the homologous but none with the heterologous species.

III. *Absorption tests*—A serum dilution of 1:50 is usually found satisfactory for absorption tests. If this does not give satisfactory results, other dilutions may be tested.

Inoculate infusion agar in two pint Blake or other bottles of convenient size with the culture homologous to the serum being standardized, and incubate at from 35 to 37° C. for from 18 to 20 hours. Suspend the growth in a small amount of 0.85 per cent salt solution, and determine its turbidity in relation to that of barium-sulphate standard No. 3. Absorbing

TABLE II

Designation of Absorbing Suspensions	*Ratio to Turbidity of $BaSO_4$ Standard No. 3*	*Dilution Necessary to Give a Suspension Equal in Turbidity to $BaSO_4$ Standard No. 3*	
		Suspension Part	*Salt Solution Part*
No. 1	Same	1	0
No. 2	2 x	1	1
No. 3	10 x	1	9
No. 4	20 x	1	19
No. 5	50 x	1	49
No. 6	100 x	1	99
No. 7	200 x	1	199
No. 8	400 x	1	399

suspensions are designated as No. 1, No. 2, etc., in Table II.

The turbidity of absorbing suspensions varies with different species and different sera. Therefore, in initial standardization, test with three or four suspensions chosen arbitrarily. Combine equal parts of the suspensions and a dilution of the antiserum which is not more than one-eightieth of its titer.

Incubate the tests at approximately 50° C. for 3 hours, inverting the tubes occasionally to insure contact of microorganisms and serum. Leave the tubes in the refrigerator over night and then centrifugalize at high speed for 1 hour, or until the supernatant fluid is clear. Prepare dilutions of the supernatant fluid and combine 0.3 c.c. of each with 0.3 c.c. of a suspension in 0.85 per cent salt solution (comparable in turbidity to barium-sulphate standard No. 3) of a beef-infusion agar culture of the species homologous to the antiserum. Incubate the tests at from 35 to 37° C. for 2 hours and leave them in the cold room over night before reading, except in the case of *B. dysenteriae* Sonne and *B. alkalescens*. Incubate tests with these species at approximately 50° C. for from 18 to 20 hours, and record the reactions. Select as a standard for use in routine tests an absorbing suspension which completely removes the agglutinative properties through at least one-twentieth of the titer of the serum.

STANDARDIZATION AND TESTING OF DIFFERENTIAL MEDIA

Whenever a new lot of dye or indicator is purchased for use in any medium, test the medium with representative strains of the bacterial species inciting enteric disease as well as *B. coli*. Also, test the efficiency of each new lot of medium.

In the case of brilliant-green agar, the amounts of dye necessary must be determined for each lot of agar base. Prepare 6 plates each of agar containing 0.1, 0.2, 0.3, 0.4, and 0.5 per cent of a 0.1 per cent aqueous solution of brilliant-green dye. Inoculate portions of each set of plates with standard strains of *B. coli*, *B. typhosus*, *B. paratyphosus* A, *B. paratyphosus* B, and *B. dysenteriae* Shiga; and, if possible, fecal specimens from cases of typhoid fever or persons known to be carriers of *B. typhosus*. After incubation for from 18 to 20 hours at from 35 to 37° C., observe the growth and select for a "weak" dye agar, that dilution which best inhibits the growth of *B. coli* without materially affecting the growth of typhoid and paratyphoid bacilli. Choose for a "strong" dye agar that dilution which inhibits the growth of *B. coli* completely or nearly so, and the growth of *B. typhosus* and *B. paratyphosus* not more than 50 per cent.

Whenever a new lot of basic fuchsin is obtained, standardize it as follows

for use in Endo's agar: To 100 c.c. amounts of agar base, add 0.05 c.c., 0.1 c.c., 0.2 c.c., and 0.3 c.c. of a saturated alcoholic solution and pour 6 plates from each. Inoculate portions of each set of plates with standard strains of *B. coli*, *B. typhosus*, *B. paratyphosus* A, *B. paratyphosus* B and *B. dysenteriae* Shiga and, if possible, fecal specimens from cases of typhoid fever or persons known to be carriers of *B. typhosus*. Examine the plates after incubation for from 18 to 20 hours at from 35 to 37° C. and select that amount of dye with which the sharpest differentiation of *B. coli* and the bacilli inciting enteric disease is obtained and which has no appreciable inhibitory action on the latter.

PREPARATION OF MEDIA AND REAGENTS

Details in regard to the preparation of media and reagents are given in the order in which they are mentioned in the preceding methods. Formulae for media in common use, such as, infusion and extract agar and broth, are not included. It is assumed that these would be available in any laboratory undertaking this type of work.

The source of dyes and chemicals which have been found satisfactory is indicated. Products which are shown to be equally reliable may be obtained from other manufacturers.

Hitchens Semifluid Agar[3]

Beef, ground	500 gm.
Distilled water	1 kg.
Peptone	10 gm.
Sodium chloride	5 gm.
Dextrose	1 gm.
Agar	1.5 gm.

Weigh the meat and add the required quantity of water. Stir thoroughly, and infuse over night in the cold room. Strain through cheesecloth, press off the juice from the meat with a meat press, and weigh. Heat gradually in a steam kettle or over a free flame, stirring occasionally, until the infusion is clear and almost colorless and the coagulum is brown. Strain through cheesecloth and filter through paper. Make up the weight to give 1 kg. of infusion for each 500 gm. of meat. Adjust the reaction to from pH 8.0 to 8.2. Add the peptone and the salt, and autoclave at 121° C. for from 30 to 40 minutes, depending on the bulk of the material. Make up the weight to that of infusion plus the other ingredients. Add the dextrose, dissolve, and filter through paper until clear.

Dissolve the agar in 50 c.c. of distilled water by heating in the autoclave at 121° C. for 30 minutes. Bring the filtered broth to the boiling point and add 40 c.c. of the melted aqueous agar per kg. Line the bottom of a Buchner funnel with two layers of filter paper cut to fit. Over these, place a thick pad of nonabsorbent cotton. Using a small amount of negative pressure and keeping the medium hot, filter it into a suction flask. Dispense in bottles or tubes in moderately tall columns and sterilize by steam at 121° C. for 20 minutes.

Conradi's Bile Medium (Modified)[4*]

Dehydrated ox bile	100 gm.
Glycerol	100 gm.
Peptone	20 gm.
Distilled water to make	1 kg.

* Slightly modified for purposes of convenience.

Combine the ingredients and dispense in from 8 to 10 c.c. amounts in wide-mouth tubes or jars. Sterilize by steam at 121° C. for 15 minutes.

Glycerol Sodium-chloride Solution[5]

Sodium chloride	6 gm.
Distilled water to make	1 kg.
Glycerol, T.P. (Schering-Kahlbaum, Berlin)	

Dissolve the salt in part of the water and make up to 1 kg. To 7 volumes of the 0.6 per cent salt solution, add 3 volumes of glycerol. Filter through paper.

Dispense in from 8 to 10 c.c. amounts in the tubes or jars used in the outfits to be distributed for the collection of specimens. Sterilize by steam at 121° C. for 20 minutes. After removing from the autoclave, allow the jars or tubes to cool to from 50 to 60° C., fasten the caps or stoppers securely, and leave standing over night in an inverted position. Discard leaky jars or tubes. Test the viability of the bacilli inciting enteric disease in each lot of glycerol solution prepared.

Eosin-methylene-blue Plating Medium [6]

Distilled water	1 kg.
Agar	20 gm.
Sodium chloride	5 gm.
Beef extract	3 gm.
Peptone	10 gm.

Dissolve the agar in about 90 per cent of the water by heating in an autoclave for 30 minutes at 121° C. Dissolve the peptone, salt, and extract in the remainder of the water by heating in a water-bath. Combine the two parts and make up the weight to that of all the ingredients. Adjust to from pH 7.0 to 7.2. Dispense in bottles of convenient size and sterilize by steam at 121° C. for from 30 to 40 minutes, depending on the volume. When plates are to be poured, melt the agar in steam at 100° C. To each 100 c.c. of medium add 5 c.c. of a sterile 10 per cent lactose solution, 1 c.c. of a 4 per cent aqueous solution of eosin, and 2 c.c. of a 0.5 per cent aqueous solution of methylene blue. Mix well before pouring into plates.

Eosin, 4 Per Cent, Aqueous Solution

Eosin (National Aniline)	40 gm.
Distilled water to make	1,000 c.c.

Dissolve the dye in part of the water and make up to volume. Store in a glass stoppered bottle.

Methylene Blue, 0.5 Per Cent, Aqueous Solution

Methylene blue (National Aniline)	5 gm.
Distilled water to make	1,000 c.c.

Dissolve the dye in part of the water and make up to volume. Store in a glass stoppered bottle.

Lactose Solution

Lactose	100 gm.
Distilled water to make	1 kg.

Dissolve the lactose in a small amount of distilled water and make up to volume. Filter through paper and dispense in convenient amounts in bottles or tubes. Sterilize by steam at 121° C. for 15 minutes.

Endo's Plating Medium—Robinson and Rettger's Modification [7, 8]

Distilled water	1 kg.
Agar	25 gm.
Peptone	10 gm.
Beef extract	5 gm.
Sodium carbonate (10 per cent solution)	10 c.c.
Lactose	10 gm.
Sodium bisulphite (10 per cent solution)	10 c.c.
Fuchsin (saturated alcoholic solution)—determined by standardization	

Add the water to the agar, reserving about 10 per cent or 100 gm. per kg. to dissolve the peptone and beef extract. Dissolve the agar by heating in an autoclave for 30 minutes at 121° C. Dissolve the peptone and extract by heating in a water-bath. Combine the two parts and make up the weight to that of all the ingredients. Filter through a thin layer of absorbent cotton. Add the solution of sodium carbonate. Check the reaction, which should be between pH 7.8 and 8.0. Heat in streaming steam at 100° C. for 10 minutes. Add the lactose and the fuchsin and mix well. Add sodium bisulphite and mix well again. Dispense in bottles in convenient amounts and sterilize by steam at from 115 to 116° C. for 20 minutes. Melt the agar and pour plates as needed.

Sodium Carbonate, 10 Per Cent Solution

Anhydrous sodium carbonate	100 gm.
Distilled water to make	1 l.

After dissolving the sodium carbonate in the water, standardize by titration with 1N hydrochloric acid.

Sodium Bisulphite, 10 Per Cent, Aqueous Solution

Sodium bisulphite	100 gm.
Distilled water to make	1,000 c.c.

Dissolve the bisulphite in part of the water in a 1,000 c.c. volumetric flask. Make up to volume. Filter through paper and use immediately or store in a wide-mouth bottle under a ¾" layer of mineral oil. Fit the bottle with a siphon and when solution is required, dispense desired amount, using the siphon.

Fuchsin, Basic, Saturated Alcoholic Solution

Fuchsin, basic (National Aniline)	10 gm.
Alcohol, 95 per cent, to make	100 c.c.

Dissolve the dye in part of the alcohol and make up to volume. Shake well. Allow to stand over night, filter through paper and store in a glass stoppered bottle.

Brilliant-green Plating Medium [9]

Distilled water	1 kg.
Agar	15 gm.
Sodium chloride	5 gm.
Beef extract	3 gm.
Peptone	10 gm.
Andrade's indicator	30 c.c.

Dissolve the agar in about 90 per cent of the water by heating in an autoclave for 30 minutes at 121° C. Dissolve the peptone, salt, and extract in the remainder of the water by heating in a water-bath. Combine the two parts and make up the weight to that of all the ingredients. Filter through a thin layer of absorbent cotton.

Weigh the filtered agar and add 3 per cent of Andrade's indicator. Determine the reaction and, if it lies between pH 7.2 and 7.6, do not adjust. Otherwise, adjust to pH 7.6. Dispense five 100 c.c. amounts in bottles for purposes of standardization. Dispense the remainder in from 100 to 400 c.c. amounts in bottles of a suitable size. Sterilize by steam at 121° C. for from 30 to 40 minutes depending on the volume. As soon as the material is removed from the autoclave, add to each of the five 100 c.c. amounts of agar 5 c.c. of a sterile solution containing 2 per cent dextrose and 20 per cent lactose. To the first bottle add 0.1 c.c., to the second 0.2 c.c., to the third 0.3 c.c., to the fourth 0.4 c.c., and to the fifth 0.5 c.c. of a 0.1 per cent aqueous solution of brilliant-green dye. (These amounts may vary with different lots of brilliant-green dye.) From each bottle pour 6 plates to be employed for standardization.

When brilliant-green agar plates are to be prepared from a lot which has been standardized, melt the agar in steam at 100° C. and add a sufficient amount of a sterile solution containing 2 per cent dextrose and 20 per cent lactose to give 0.1 and 1 per cent respectively, of these cabohydrates. Add the required amounts of 0.1 per cent aqueous solution of brilliant green as determined by standardization.

Andrade's Indicator [10, 11]

Acid fuchsin (Coleman and Bell)	
Distilled water	1,000 c.c.
1N NaOH	100 c.c.

It is necessary to test each lot of fuchsin purchased. Make up a small amount of indicator using the same amount of fuchsin as was used with the preceding lot for the preparation of the indicator (from 1.5 to 5 gm. per l.). Incorporate this indicator in a very small amount of double-sugar medium and test it with cultures of the bacterial species inciting enteric disease. If the uninoculated medium of suitable reaction is decidedly pink when cold, less fuchsin should be used.

Lactose-dextrose Solution

Dextrose	20 gm.
Lactose	200 gm.
Distilled water to make	1 kg.

Dissolve the carbohydrates in a small amount of distilled water and make up to volume. Filter through paper and dispense in convenient amounts in bottles or tubes. Sterilize by steam at 121° C. for 15 minutes.

Brilliant Green, 0.1 Per Cent, Aqueous Solution

Brilliant green (Coleman and Bell)	0.5 gm.
Distilled water to make	500 c.c.

Dissolve the dye in part of the water and make up to volume. Store in a glass-stoppered bottle in the dark.

Russell's Double-sugar Medium [12]

Distilled water	1 kg.
Agar	15 gm.
Sodium chloride	5 gm.
Beef extract	3 gm.
Peptone	10 gm.
Andrade's indicator	30 c.c.
Lactose	10 gm.
Dextrose	0.5 gm.

Dissolve the agar in about 90 per cent of the water by heating in an autoclave for 30 minutes at 121° C. Dissolve the peptone, salt, lactose, dextrose, and extract in the remainder of the water by heating in a water-bath. Combine the two parts and make up the weight to that of all the ingredients. Filter through a thin layer of absorbent cotton.

Weigh the filtered agar and add 3 per cent of Andrade's indicator. Determine the reaction and, if it lies between pH 7.2 and 7.6, do not adjust. Otherwise adjust to pH 7.6. Dispense in tubes and after sterilization by steam at 121° C. for 12 minutes, cool in a slanted position so that the agar will form a butt about 1″ deep and a slant from 1.5 to 2″ long.

Peptone Water with Carbohydrate [13]

(For determination of acid production)

Distilled water	1 kg.
Peptone	10 gm.
Sodium chloride	5 gm.
Andrade's indicator	10 c.c.

Add the peptone and salt to the water, and dissolve, warming if necessary. Filter through paper. Add the indicator and adjust to from pH 7.4 to 7.5. Dispense in convenient amounts in bottles and sterilize by steam at 121° C. for 20 minutes.

Add sterile solutions of carbohydrates to give 1 per cent and dispense in from 1 to 2 c.c. amounts in small tubes, using precautions to avoid contamination. Solutions of carbohydrates sterilized by filtration should be employed, except in the case of those which are relatively stable at high temperatures. Seal with paraffin to prevent evaporation.

Beef-extract Broth with Carbohydrate [14]

(For determination of gas production)

Distilled water	1 kg.
Beef extract	3 gm.
Peptone	5 gm.
Carbohydrate	5 gm.

To the required quantity of water add the extract and peptone. Dissolve by heating over a free flame, stirring frequently. Adjust to pH 7.0. Boil for about 5 minutes. Make up lost weight. Add the sugar, dissolve, and filter through paper until clear. Dispense in Durham fermentation tubes with outer tube 150 by 19 mm. and inner tube 75 by 11 mm. in size. Fill a small amount of broth (about 1.5 cm. depth) into the large tube, fill the small tube completely and invert in the large tube. Sterilize by steam at 121° C. for 12 minutes.

Dunham's Peptone Solution (Modified) [15]

(For determination of indol production)

Distilled water	1 kg.
Peptone (Parke-Davis)	10 gm.
Potassium nitrate C.P.	0.1 gm.

Dissolve the peptone in the water, warming if necessary, and adjust to pH 7.0. Add the nitrate, and filter through paper. Dispense in from 5 to 6 c.c. amounts in tubes and sterilize by steam at 121° C. for 15 minutes.

Parke-Davis peptone, or some other brand known to be satisfactory for indol production, must be used.

Ehrlich's Reagent

(For determination of indol production)

Paradimethylamino benzaldehyde (Pfanstiehl) . . .	1 gm.
Absolute alcohol	95 c.c.
Concentrated hydrochloric acid	20 c.c.
Store in a glass-stoppered bottle.	

This reagent should be used within 2 months after preparation.

Litmus Milk

Milk, certified or Grade A, unpasteurized	1 quart
Litmus solution, 10 per cent, aqueous	2 per cent by volume

Pour the milk into a separatory funnel and place in the refrigerator over night to allow the cream to rise. Draw off the fat-free milk and add the required volume of litmus solution. If necessary, adjust the reaction to neutral to the indicator. Dispense in from 5 to 6 c.c. amounts in tubes and sterilize by steam at 100° C. for 20 minutes on 3 successive days.

Litmus Solution

Purified litmus	10 gm.
Distilled water to make..	100 c.c.

Grind the litmus in a mortar. Transfer to a 100 c.c. volumetric flask, add about one-half of the water, and shake vigorously. Allow to stand and shake again. Repeat. Finally add sufficient water to make up to volume. Allow to settle over night, decant the clear solution, and centrifugalize until clear. Add 0.5 per cent chloroform and store in a glass-stoppered bottle in a refrigerator.

Purified litmus may be purchased or may be prepared [16] as follows:

Commercial litmus	20 gm.
Alcohol, 85 per cent	500 c.c.
(445 c.c. of 95 per cent alcohol diluted to 500 c.c.)	

Powder the litmus in a mortar. Transfer to an evaporating dish and add 100 c.c. of alcohol which has been heated to boiling on a steam bath. Place the evaporating dish in the steam bath and digest with frequent stirring for 15 minutes. Centrifugalize and discard the alcoholic supernatant solution. Extract the precipitate in this manner 5 times. Transfer the extracted litmus to a shallow dish and dry in a vacuum desiccator over concentrated sulphuric acid.

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MARION B. COLEMAN, *Referee*

Tentative Methods for the Isolation and Identification of Hemolytic Streptococci*

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References

As aids to diagnosis, laboratory methods depend upon definite knowledge of the etiological relationship of a microörganism to a disease process. That hemolytic streptococci incite many quite different and characteristic infectious processes is well established, but no specific relationship of any of the various streptoccoci to a particular disease has as yet secured general acceptance. Knowledge of the biological characters, however, has advanced steadily and to a stage of real, though limited, usefulness for the isolation and identification of hemolytic streptococci. Certain difficulties arise in the practical

* Report of the Referee on Recognition and Significance of Hemolytic Streptococci in Infectious Diseases for the Standard Methods Committee on Diagnostic Procedures and Reagents.

application of this knowledge because of the variety of procedures and the lack of essential information in the literature. Therefore, as a preliminary step in the standardization of methods, tentative procedures which have proved practical in actual use for the preparation and examination of specimens for hemolytic streptococci and for their identification have been formulated; they are submitted for study and criticism.

General Directions

I. Preparation of Specimen for Examination

A. Specimens are collected in sterile glass containers, stoppered or sealed to prevent leakage and contamination. Each specimen container bears a label on which is written the patient's name and, if sent to the laboratory by mail, it is packed in such a way as to comply with the postal regulations. Specimens of milk are kept iced until examined. If this is impossible, one volume of glycerol [1] — Schering Kahlbaum — is mixed thoroughly with two volumes of milk as soon as the sample is taken.

B. Each specimen is accompanied by a history slip on which the following data are recorded: kind of specimen and date of collection, identification and pertinent clinical history of the patient or subject, and the name and address of the physician.

C. Specimens are opened and numbered one at a time in order to avoid the possibility of interchanging them. This number is recorded on the specimen label and on the history slip. The records of all examinations made of the specimen are marked with this number.

II. Apparatus and Technic for Examination

A. All glassware, solutions, and media are sterile and handled with the usual precautions to prevent contamination.

B. Incubators are maintained at a temperature of from 34 to 36° C. and are equipped with thermometers which register maximum and minimum temperatures.

C. Solid media are inoculated with a wire loop. Plate cultures are made by streaking one loopful (1 mm.) of material in parallel rows with particular care to avoid breaking the surface of the medium. Liquid media are inoculated by adding with a pipette suitable volumes of 18 hour broth culture.

D. With the following exceptions, inoculated media are incubated under aerobic conditions: blood-agar plates used for isolation and fishings from these plates to broth of strains which fail to grow aerobically.

E. Preparations for microscopic examination are made by spreading a loopful of material evenly on a glass slide and are stained by Gram's method. Films of *B. coli* and of *Staphylococcus aureus* are placed on each slide as controls of the staining procedure. When pus cells are present, the staining reactions of their nuclei serve as an excellent control.

F. Seed cultures for the inoculation of the various test media are incubated for 18 hours and examined microscopically before use, and are streaked on blood agar after use to assure their purity. If any evidence of contamination is noted, the differential tests are repeated with cultures freed from contaminants by plating and fishing.

Isolation

I. Media *

A. Blood agar [2] for plates and slants: Preparation—

Beef heart, ground.....	1 kg.
Distilled water	2 kg.
Agar, shredded No. 40, A. H. Thomas Co.	40 gm.
Sodium chloride, C.P.....	10 gm.
Peptone, "Difco" Bacto...	10 gm.

* The preparation of the different media is still under investigation, with a view to simplifying the procedures.

Mix the meat and 1 kg. of water thoroughly and infuse over night at 5° C. Skim the fat and heat the infusion to 45° C., hold at between 45 and 50° C. for 1 hour, and then boil for ½ hour. Stir frequently throughout the heating process. Remove the coarse particles by straining through a colander, and filter through glass wool which has been washed thoroughly in distilled water. Add distilled water to make 1 kg. and adjust the infusion to pH 7.4. Boil for 5 minutes and filter again through glass wool and make up any loss in weight with distilled water.

Dissolve the agar and sodium chloride in 1 kg. of water by heating in an autoclave at 121° C. for 30 minutes. Mix equal parts of this solution and the infusion with the peptone and adjust the reaction to pH 7.4 if necessary. Filter through glass wool, dispense the medium in suitable quantities, and sterilize by steam at 121° C. for 30 minutes.

Melt 900 c.c. of the beef-heart-infusion agar, cool to 45° C., and add 50 c.c. of sterile defibrinated horse, rabbit, or human blood. Dispense in tubes or Petri plates. After solidification, leave at room temperature for 48 hours, and examine for contamination. Store at 5° C.

Testing—Test each lot of medium by inoculating plates with several strains of streptococci of known hemolytic activity; include in this test at least one strain, the hemolytic activity of which is inhibited by the presence of small quantities of dextrose in the medium. Incubate under aerobic conditions, and examine for hemolysis after 24 hours. Do not use the medium unless all control strains induce distinct hemolysis.

B. Beef-infusion agar, 0.12 per cent,[3] *for blood cultures:*

Beef, ground	500 gm.
Distilled water	1 kg.
Peptone, "Difco" Bacto	10 gm.
Sodium chloride, C.P.	5 gm.
Dextrose, anhydrous C.P., Merck	1 gm.
Agar, 3 per cent aqueous	40 c.c.

Mix the meat and water thoroughly and infuse over night at 5° C. Strain through cheesecloth and press the juice from the meat in a meat press. Heat to 45° C. in a water-bath and hold at that temperature for 1 hour. Boil for 50 minutes, stirring occasionally, and filter through glass wool which has been washed thoroughly in distilled water. Add distilled water to bring weight to that of the water added originally, and adjust to from pH 8.0 to 8.2. Dissolve the peptone, salt, and dextrose in the infusion. Filter through glass wool until clear. Warm the filtrate, add the melted agar, and filter through glass wool if necessary. Dispense and sterilize by steam at 121° C. for 30 minutes.

C. Beef-infusion broth[4]:

Beef, ground	500 gm.
Distilled water	1 kg.
Peptone, "Difco" Bacto	10 gm.
Sodium chloride, C.P.	5 gm.

Mix the meat thoroughly with approximately 90 per cent of the water, reserving the remainder to dissolve the peptone and salt. Infuse over night at 5° C., strain the infusion through cheesecloth, and press the juice from the meat with a meat press. Dissolve the peptone and salt in the water by heating in a water-bath, and add to the infusion. Adjust the reaction to neutral to phenolphthalein. Boil for 30 minutes. Check the reaction and, if it is more acid than pH 8.0, adjust and boil for 3 minutes. Filter through paper and make up weight with distilled water to the total of all ingredients, excluding the meat. Dispense and sterilize by steam at 100° C. for 40 minutes on 3 successive days. Incubate for 48 hours, and examine for contamination before use.

II. PLATING OF SPECIMEN

Plate each specimen in duplicate on blood-agar medium (see Isolation I A)

by streaking the surface of each plate with one loopful of material. Incubate one plate under anaerobic conditions in a hydrogen jar [5] containing as a control a tube of methylene-blue indicator* and examine for growth after 48 hours; incubate the other under partial carbon-dioxide tension † and examine after 24 hours; if no growth of hemolytic streptococci is present, examine again after 48 hours.

A. Blood:

Keidel tube—Open the tube in a flame and with a bulb Pasteur pipette or a 20 c.c. syringe (needle, 17 gauge, 1½"), transfer approximately 15 c.c. of the contents to a test tube. From this material make a slide preparation; transfer from 3 to 5 c.c. to each of two 100 c.c. bottles containing 45 c.c. of 0.12 per cent beef-infusion agar which has been warmed to 37° C., and streak a loopful on each of two blood-agar plates. If no growth is present on these plates after incubation, make stained preparations and inoculate plates from the 0.12 per cent beef-infusion-agar cultures after 2, 7, and 14 days' incubation.

If the patient is available to the laboratory, inoculate bottles of 0.12 per cent beef-infusion-agar medium with the freshly drawn blood and proceed as indicated above.

Clotted blood—Pipette the serum from the clot without centrifugalization and place the clot in a 200 c.c. bottle containing 110 c.c. of 0.12 per cent beef-infusion agar which has been warmed to 37° C., and incubate. Make a slide preparation from and plate on blood agar the material left in the tube after the removal of the clot. If no growth is present on these plates after incubation, make stained preparations and inoculate plates from the 0.12 per cent beef-infusion-agar culture after 2, 7, and 14 days' incubation.

B. Nose and throat cultures:

Loeffler's coagulated serum medium—If the specimen consists of an inoculated slant of Loeffler's coagulated serum medium, incubate for from 18 to 24 hours. Make a slide preparation by passing a loop lightly over the entire surface of the medium and spreading the material evenly in a drop of water on a slide. Suspend a representative loopful of culture in 2 c.c. of 0.85 per cent salt solution and plate on blood agar.

If the swab with which the culture was taken is returned with Loeffler culture, treat as described below.

Swab—Wash the swab in 2 c.c. of 0.85 per cent salt solution, and streak both the moist swab and the washings on one half of each plate. Make stained preparations from both the swab and its washings.

If the patient is available to the laboratory, inoculate the blood-agar plates with the swab at the bedside. Return plates to the laboratory for incubation and examination. Also return the swab and, if no growth of hemolytic streptococci occurs on the plates inoculated at the bedside, examine the swabs as indicated above.

C. Purulent discharges:

Tube specimen—Make a stained preparation and plate on blood agar. If the examination of stained preparation indicates the presence of a large number of microörganisms, dilute a portion of the material with 0.85 per cent salt solution before plating.

Swab—Proceed as in the examination of swabs—nose and throat cultures.

D. Spinal fluid:

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* Methylene-blue indicator: Mix in a test tube equal parts of N/160 sodium hydroxide, 0.015 per cent aqueous methylene blue, and 6 per cent sterile aqueous solution of dextrose. Boil until the methylene blue is reduced, and place in the hydrogen jar immediately before it is sealed for the removal of the oxygen. The indicator remains colorless as long as anaerobic conditions are maintained.

† Place the inoculated plates in a 2½ liter museum jar or other container of similar volume which can be sealed tightly. Put a tube containing approximately 0.6 gm. of sodium carbonate in the jar in an upright position and with a pipette add 10 c.c. of 10 per cent sulphuric acid. Seal the jar as soon as the reaction begins to subside.[6]

Centrifugalize at approximately 2,000 r.p.m. for ½ hour, remove the supernatant fluid, and make a stained preparation from the sediment. Draw the remainder of the sediment into a fine capillary pipette, place 1 drop on each plate, and streak with a wire loop. If microscopic examination of the stained preparation indicates the presence of Gram-positive cocci, inoculate also a tube of beef-infusion broth (see Isolation I C).

E. Milk:

Centrifugalize at approximately 2,000 r.p.m. for ½ hour to separate the cream. Draw off the skim milk with a capillary pipette, and discard. Make a stained preparation and plate on blood agar the mixed cream and sediment.

If haste is not required, store the milk specimen at 5° C. until the cream has separated. Make a stained preparation and plate the gravity cream.

III. FISHING COLONIES

Examine the plates thoroughly after incubation for isolated hemolytic colonies of characteristic appearance. Fish several colonies, transferring part of the growth from each colony to a blood-agar slant and a tube of beef-infusion broth* (see Isolation I C). If hemolytic colonies are present, but are overgrown, suspend some of the growth from these areas in 0.85 per cent salt solution, and replate. If the growth is heavy—few or no isolated colonies—dilute a portion of the specimen with 0.85 per cent salt solution and replate.

Incubate fishings to blood-agar slants until growth is obtained, usually 24 hours, examine the growth macroscopically for homogeneity and hemolytic activity, and store at 5° C. for further study if necessary.

After incubation, examine fishings to broth microscopically for the presence of Gram-positive cocci which form chains. Plate on blood agar and incubate under aerobic conditions. Examine the growth on the plates for homogeneity and hemolytic activity.

IDENTIFICATION

The extent to which the procedures for identification and differentiation are carried is determined by the type of specimen and the purpose of the examination. For microörganisms from specimens of blood and spinal fluid and, in many instances, purulent discharges, a study of the morphology and hemolytic activity alone suffices to establish the etiological relationship. With strains from milk and from nose and throat cultures, particularly when they are examined in connection with the investigation of outbreaks of streptococcus infections, however, additional data are essential.

The presence in the normal nose and throat, and in the udders of cows, of hemolytic streptococci, usually considered of little importance as incitants of infectious disease in man, has necessitated the formulation of procedures by which the common human pathogens may be differentiated. In general, these strains are hemolytic not only when grown on blood agar but also when examined by means of the tube test. They also produce a pH of 4.6 or above in 1 per cent dextrose broth, ferment trehalose, fail to ferment sorbitol, or to hydrolyze sodium hippurate, and, according to the results of precipitation tests, are members of Group A described by Lancefield.

I. TUBE TEST FOR HEMOLYSIS [7, 8]

Add 0.5 c.c. of a 15 hour beef-infusion broth (see Isolation I C) culture to 0.5 c.c. of 5 per cent horse, rabbit, or human red-blood cells washed 3 times in 0.85 per cent salt solution. Mix

* If a strain is encountered fishings of which fail to grow in this medium, add to the broth approximately 10 per cent of normal serum or 5 per cent of defibrinated blood.

thoroughly and incubate in a water-bath for 2 hours at 37° C. Examine for hemolysis after ½, 1, and 2 hours. For purposes of control, include two tubes, one containing 0.5 c.c. of uninoculated broth mixed with 0.5 c.c. of cells, and the other containing 0.5 c.c. of culture of a known hemolytic strain and 0.5 c.c. of cells. At the end of the 2 hour incubation period, centrifugalize any tubes in which hemolysis appears incomplete or is absent, and compare them with the uninoculated broth control.

II. DIFFERENTIAL TESTS FOR THE IDENTIFICATION OF STRAINS COMMONLY ENCOUNTERED IN HUMAN INFECTIONS

A. Final hydrogen-ion concentration [9, 10]:

Medium: Dextrose beef-infusion broth—

Beef, ground	500 gm.
Distilled water	1 kg.
Peptone, "Difco" Proteose	20 gm.
Sodium chloride, C.P.	5 gm.
Dextrose, anhydrous C.P., Merck, 20 per cent solution	50 c.c.

Mix the meat and water thoroughly and infuse over night at 5° C. Strain through cheesecloth and press the juice from the meat in a meat press. Boil for 30 minutes, stirring occasionally. Strain through cheesecloth and filter through paper. Dissolve the peptone and salt in the filtrate and make up weight with distilled water to the sum of all the ingredients, excluding the meat. Adjust the reaction to pH 8.2 and heat at 121° C. for from 30 to 40 minutes, depending on the bulk of the material. Make up weight, and check the reaction. If it is more acid than pH 8.2, readjust and boil for 3 minutes. Filter through paper until clear and sterilize by steam at 121° C. for 20 minutes.

Add the heat-sterilized 20 per cent dextrose solution to the medium aseptically and dispense in 5 c.c. amounts in 125-by-13-mm. tubes. Incubate the finished medium for 2 days and examine for contamination.

Procedure—Inoculate a tube of dextrose beef-infusion broth with 0.2 c.c. of broth culture, and incubate for 96 hours. Determine the hydrogen-ion concentration colorimetrically.

Colorimetric determination of hydrogen-ion concentration—Pipette 2 c.c. of freshly boiled and cooled distilled water and 1 c.c. of the supernatant fluid from dextrose-broth culture into each of two clear hard-glass tubes, 75-by-11-mm. To one of these add 0.1 c.c. of methyl red [11, 12] * or other indicator of suitable range, and mix thoroughly the contents of both tubes. In a comparator block superimpose the tube containing the diluted culture and indicator upon a tube of distilled water; superimpose the other tube upon a color standard prepared by adding 1 c.c. of standard buffer solution [13, 14] of suitable hydrogen-ion concentration and 0.1 c.c. of indicator to 2 c.c. of distilled water. Record as the hydrogen-ion concentration of the culture that of the standard which it most nearly matches in color. (Because of the instability of methyl-red indicator, fresh "standards" should be prepared daily.)

B. Hydrolysis of sodium hippurate [15]:

Medium: Sodium-hippurate pepsin broth—[16]

Distilled water	1 kg.
Peptone, Parke-Davis Bacteriologic	10 gm.
Pepsin, U.S.P., Eimer and Amend	5 gm.
Calcium chloride, C.P.	0.03 gm.
Ferric chloride, C.P., Baker, 1 per cent solution	1 drop
Sodium hippurate, C.P., Eimer and Amend	5 gm.

Dissolve all the ingredients except the sodium hippurate in the water, divide in two equal parts, and adjust one to

* Methyl red is prepared according to the procedure given in the reference to Standard Methods, except that 60 per cent alcohol is used in place of distilled water for the final dilution.

pH 7.1. To the other part—500 gm.—of medium, add the sodium hippurate and adjust to pH 7.1. Boil both for 2 minutes, add distilled water to compensate for loss in weight, and filter through paper. Dispense in 5 c.c. amounts in 125-by-13-mm. tubes and sterilize by steam at 121° C. for 20 minutes.

Procedure—Inoculate a tube of sodium-hippurate pepsin broth with 0.2 c.c. of broth culture and incubate for 48 hours, or longer if growth is poor. Inoculate and incubate similarly a tube of the same medium to which no sodium hippurate has been added. To 2 c.c. of these cultures add 0.5 c.c. of 12 per cent ferric-chloride solution, prepared by dissolving the salt in distilled water containing 2 c.c. of concentrated hydrochloric acid per liter. Mix thoroughly and let stand for from 5 to 10 minutes before recording the presence or absence of hydrolysis—presence indicated by the formation of a heavy precipitate. With each series of cultures tested, include as controls two strains, one of which does and the other of which does not hydrolyze sodium hippurate.

C. Fermentation of trehalose and sorbitol [17, 18]:

Medium: 3 per cent chlor-phenol-red beef-infusion broth—

Beef-infusion broth (see Identification IIA, *without* dextrose)	100 c.c.
Chlor-phenol-red [11, 12]	3 c.c.
Trehalose, "Difco" standardized, 10 per cent solution, sterilized by filtration	10 c.c.
or	
d-Sorbitol, "Difco" standardized, 10 per cent solution, sterilized by heat	10 c.c.

Add the indicator to the sterile broth and heat by steam at 100° C. for 20 minutes. Cool and add either trehalose or sorbitol aseptically and dispense in approximately 2 c.c. amounts in 75-by-11-mm. tubes. Incubate for 48 hours, and examine for contamination. For control purposes dispense similarly an equal number of tubes of the medium containing the indicator but no added carbohydrate.

Procedure—Inoculate a tube of 3 per cent chlor-phenol-red beef-infusion broth containing each of the test substances with 0.1 c.c. of culture. As a control on the amount of natural sugar in the medium, inoculate similarly a tube of the medium to which no carbohydrate has been added. Incubate for 1 week and record the presence or absence of fermentation after 1, 2, and 7 days.

D. Group-precipitation test [19]:

1. Extracts of strains

Medium: Infusion-free peptone broth —[20, 21]

Sodium chloride, C.P.	5 gm.
Dipotassium phosphate, C.P.	1 gm.
Monopotassium phosphate, C.P.	1 gm.
Peptone, Parke-Davis Bacteriologic	20 gm.
Cane sugar	2 gm.
Distilled water to make	1 kg.

Dissolve the sodium chloride in part of the water, add peptone, and heat slightly to dissolve. Dissolve the potassium salts separately in small quantities of water, and add to the peptone-salt solution. Add water to make 1 kg., and adjust to pH 7.8. Heat at 121° C. for 40 minutes, add cane sugar, and make up with water any loss in weight. Filter through paper until clear, dispense in 450 c.c. amounts in 1 liter Erlenmeyer flasks, and sterilize by steam at 121° C. for 30 minutes.

Procedure — Inoculate infusion-free peptone broth* with approximately 8 c.c. of broth culture, and incubate for 48 hours. Examine a stained preparation and streak on a blood-agar plate. If microscopic examination reveals no evidence of contamination, separate the bacterial cells by centrifugalization, and discard the supernatant broth. Wash

* For certain strains which fail to grow in this medium, beef-infusion broth (see Identification IIA, containing 0.02 per cent dextrose) is used.

the cells by suspending them in 25 c.c. of 0.85 per cent salt solution and centrifugalize. After discarding the salt-solution washings, mix with the sediment 4.5 c.c. of 0.85 per cent salt solution containing sufficient N/1 hydrochloric acid to make a final concentration of N/20 hydrochloric acid. Heat in a boiling water-bath for 20 minutes. Cool under running water, centrifugalize, pipette off the supernatant fluid, and discard the sediment. Adjust the supernatant fluid with N/1 sodium hydroxide to from pH 7.0 to 7.2 by the spot-plate method.* Centrifugalize and pipette off the clear supernatant fluid for use in the test if a pure culture of hemolytic streptococci grows on the blood-agar plate inoculated with the infusion-free peptone broth culture.

2. Immune sera

Preparation of formalin-treated culture—Inoculate a suitable volume, from 50 to 100 c.c., of broth (see Identification II A, containing 0.02 per cent dextrose) and incubate for 18 hours. Examine a stained preparation, and streak on a blood-agar plate. If no evidence of contamination is obtained from the examination of the stained preparation, separate the bacterial cells from the broth by centrifugalization and resuspend in one-twentieth the volume of 0.85 per cent salt solution containing 0.25 per cent formalin. Store at 5° C. for 48 hours and, if no contaminants are noted in the plate culture, plate the formalinized suspension on blood agar. Retest after further storage if growth occurs. Dilute formalin-treated culture with 0.85 per cent salt solution to the original volume of the culture immediately before each inoculation.

Immunization of rabbits—Inoculate rabbits weighing approximately 2,000 gm. with formalin-treated cultures of strains representative of the various groups. Inject intravenously 1 c.c. of culture daily on 6 successive days, and take trial bleedings 5 days after the 6th inoculation. Test sera with extracts of homologous and heterologous strains which represent types belonging to the same group. Administer additional series of injections as indicated from the results of tests of trial bleedings, and bleed out animals from the 5th to the 7th day following the last injection.

3. Precipitation test

Pipette 0.1 c.c. of undiluted serum into 100-by-8-mm. tubes and add without mixing an equal volume of the extract* to be tested. Include as controls tubes containing similar volumes of each extract and 0.85 per cent salt solution, of 0.85 per cent salt solution and each serum, and of homologous extract and serum. Record the presence of ring formation after the test has stood for 10 minutes at room temperature or in a water-bath at 37° C. If no ring formation occurs with any of the group sera, incubate for 2 hours in a water-bath at 37° C. and store over night at 5° C. before recording the final results.

REPORTING RESULTS

A written report on the examination of each specimen is sent to the physician who submitted the specimen. The presence of hemolytic streptococci is reported as soon as the microörganisms are isolated in pure culture. In those instances in which the differential tests are performed, this report is followed by one recording the results of further procedures for identification.

Report " Hemolytic streptococci were found " or " were not found," depending upon whether or not Gram-positive cocci

* Place one loopful of phenol red 11, 12 in each of several depressions in a porcelain reaction plate. With a 0.2 c.c. pipette add 0.04 c.c. of the adjusted extract, mix thoroughly, and compare with the color standards prepared by mixing similar amounts of indicator and standard buffer solutions of pH 7.0 and 7.2 respectively. Add N/1 sodium hydroxide or N/1 hydrochloric acid as indicated. (From 0.12 to 0.14 c.c. of N/1 sodium hydroxide is usually required to adjust 4.5 c.c. of the extract.)

* Depending upon the potency of the immune sera, it may be necessary to increase or decrease the amount of antigen used in the test.

which form chains and hemolyze red-blood cells are isolated.

Report "Hemolytic streptococci were found which belong to the group commonly encountered in human infections" when hemolytic streptococci are isolated which have a final pH of 4.6 or higher in 1 per cent dextrose broth, ferment trehalose, fail to ferment sorbitol, and to hydrolyze sodium hippurate and which, according to precipitation tests, are members of Group A.

Report "Hemolytic streptococci were found which differ in certain of their serological properties from those commonly encountered in human infections" when hemolytic streptococci are isolated which do not belong to Group A according to precipitation tests. Such strains may be similar to or differ from the common human pathogens in biochemical properties.

SPECIAL TESTS

In the investigation of outbreaks of hemolytic streptococcus infections, especially those in which epidemiological data point to a carrier as the source of the incitant, differentiation of members of the group commonly encountered in human infections is occasionally essential. Until the practical value of various serological procedures for the typing of strains [22, 23] is established, the demonstration of differences in their biochemical and toxigenic properties may yield helpful information. Furthermore, the study of the toxigenic characters may prove of very practical value, not only in the control of epidemics but also in the treatment of cases, if and when the hemolytic streptococci are classified according to toxin group and thus in relation to the antitoxic or antibacterial sera, rather than by disease entity.

I. FERMENTATION OF LACTOSE, MANNITOL, AND SALICIN [24]

Medium: 3 per cent chlor-phenol-red beef-infusion broth—

Beef-infusion broth *without* dextrose	100 c.c.
Chlor-phenol-red [11, 12]	3 c.c.
Lactose, "Difco" standardized, 10 per cent solution sterilized by filtration	10 c.c.
Mannitol, Merck, 10 per cent solution sterilized by heat	10 c.c.
Salicin, U.S.P., Merck, 10 per cent solution sterilized by filtration	10 c.c.

Prepare the medium as described under Identification IIC, substituting lactose, mannitol, or salicin for the trehalose or sorbitol.

Procedure (see Identification II C)—Test for toxin production strains which have not been differentiated by this test.

II. TOXIGENIC PROPERTIES [25, 26, 27]

A. Preparation of filtrates:

Medium: Beef-infusion broth (see Identification II A) containing 0.02 per cent dextrose.

Procedure—Inoculate a 50 c.c. Erlenmeyer flask containing 20 c.c. of broth and incubate for 48 hours. If the examination of a stained preparation reveals characteristic morphology, plate on blood agar, add 0.5 per cent phenol—C. P., Baker—and sterilize by passage through a Seitz or Berkefeld filter. Store at 5° C. If no contaminants are noted on the blood-agar plate, test the filtrate by the intracutaneous inoculation of susceptible rabbits.

B. Test for toxin production:

Animal—Select white-skinned rabbits—weight, at least 3,000 gm.; age, 1 year or older—and remove the hair from a small area on one flank with electric clippers. Dilute the standard toxin with 0.85 per cent salt solution so that 0.1 c.c. of the diluted material contains 5 skin test doses of toxin. Heat a portion of the diluted toxin for 2 hours in a boiling water-bath and inject 0.1 c.c. of the heated and of the unheated material intracutaneously in the depilated area on the rabbit. Examine the rabbit

24 hours after inoculation and record the size of the reaction and the degree of redness and swelling at the site of each injection. For the determination of toxigenic activity, it is necessary to use rabbits in which 5 skin test doses of the standard toxin induce definite reactions of approximately 2 cm. in diameter and in which the heated toxin fails to react.

Procedure—Make a 1:100 dilution in 0.85 per cent salt solution of the filtrate of the strain to be tested and heat a portion of the dilution for 2 hours in a boiling water-bath. With a 1 c.c. tuberculin syringe (needle, 26 gauge, ⅜"), inject 0.1 c.c. of the heated filtrate, followed by 0.1 c.c. of the unheated material, intracutaneously in separate areas of the depilated back or sides of a susceptible rabbit. On the same animal inject 5 skin test doses of standard toxin, heated and unheated. After 24 hours, record the size of the reaction and the degree of redness and swelling at the site of each inoculation. If 0.001 c.c. of unheated filtrate (0.1 c.c. of 1:100 dilution) induces a reaction measuring 1 cm. or more in diameter and the heated filtrate fails to react, consider the strain toxigenic.

If the strains cannot be differentiated by their ability or failure to produce toxin, test the filtrates of the toxigenic strains for neutralization by antistreptococcus serum.

C. Test for neutralization by antistreptococcus serum:

Prepare dilutions of standard toxin in 0.85 per cent salt solution so that 5 skin test doses are contained in 0.1 and 0.05 c.c. respectively. Divide the first dilution in two parts and heat one for 2 hours in a boiling water-bath. Add the second dilution to the standard antistreptococcus serum so diluted with physiological salt solution that 0.05 c.c. contains sufficient serum to neutralize from 5 to 10 skin test doses of homologous toxin.

Make dilutions of 1:100 and 1:50 of the toxin to be tested and heat a portion of the 1:100 dilution for 2 hours in a boiling water-bath. Add the 1:50 dilution to an equal volume of serum diluted as for the control toxin-serum mixture.

After the toxin-serum mixtures have been allowed to stand at least ½ hour at room temperature, inject 0.1 c.c. of each intracutaneously in a susceptible rabbit. Inoculate the same animal with 0.1 c.c. amounts of heated and unheated toxins and of serum alone, so diluted that 0.1 c.c. contains sufficient serum to neutralize from 5 to 10 skin test doses of homologous toxin. Use a different 1 c.c. tuberculin syringe (needle, 26 gauge, ⅜") for the injection of the serum control, for the toxin-serum mixtures, and for the heated and unheated toxins. After 24 and 48 hours record the size of the reaction and the degree of redness and swelling at the site of each inoculation.

Consider the test toxin neutralized by the antistreptococcus serum under the following conditions: no reactions at the site of the injections of heated toxins, of serum alone, and of mixtures of serum and test and standard toxins; reactions more than 1 cm. in diameter induced by both test and standard toxins.

For further information concerning the identification and differentiation of the streptococcus toxins, consult references 25, 26, and 27 in the bibliography.

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JULIA M. COFFEY, *Referee*

Tentative Methods for the Diagnosis of Meningococcus Meningitis and Identification of the Meningococcus*

A. Meningococcus defined
B. Distribution of meningococci
C. Diagnostic procedures
  a. Cerebrospinal fluid
    1. Collection of specimen
    2. Cell counts
    3. Estimation of sugar in
    4. Blood sugar in relation to
    5. Stained smears
    6. Culture methods for isolation and identification of meningococci
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DEFINITION

It is necessary, first of all, to define what is meant by a meningococcus in order to establish definite criteria for its identification. Such a definition should include only such characteristics as are essential for identification. Reducing these to their very lowest terms we may, for practical purposes, begin with the following:

> The meningococcus is a Gram-negative coccus, usually occurring in pairs with flattened adjacent sides, which ferments dextrose and maltose with the production of acid.

The cocci may occur singly and sometimes in groups, and the individual cells may vary greatly in size and in intensity of staining, though they are always Gram-negative. Strains that fail to ferment maltose or dextrose, or both of these sugars, may be encountered. Such strains usually acquire this property sooner or later, though it may be lacking at a time when it is desired to make a diagnosis of meningococcus infection. In our recent studies of 610 strains of meningococci we have found no strain that has failed to ferment these two sugars at some time during our investigations.

Opinions as to the adequacy of the above brief definition will not be unanimous. Four other characteristics would be included by many workers with meningococci:

> Characteristic colony formation, lack of pigment production, growth only at incubator temperatures, and agglutinability by polyvalent anti-meningococcus serum.

As a rule the smooth translucent colonies of the meningococcus are quite characteristic, but occasional strains of true meningococci are found that immediately after isolation may be as large as a dime, very convex, or even opaque.

Most are agreed that meningococci never produce yellow pigment. The only dissenting voices are of those who consider *Neisseria flavescens* [1] to be a form of meningococcus,[2, 3] though both culturally and serologically *N. flavescens* seems quite distinct.

That meningococci grow only at incubator temperature is, as a rule, a safe generalization to make. We have

* Report of the Referee on the Diagnosis of Meningococcus Meningitis and the Identification of the Meningococcus for the Standard Methods Committee on Diagnostic Procedures and Reagents.

encountered a few strains that grow well at room temperature, and have had 3 trains, typical meningococci in all other respects, that were maintained for nearly a year by transplanting, incubating, and storing entirely at 20° C.

Some strains are inagglutinable when they are first recovered from patients. Practically all of these can be coaxed to agglutinate later, though they do not do so in time to supply a criterion for diagnosis. Many experienced workers do not consider agglutination necessary for identification of the meningococcus.

A more complete definition of the meningococcus would therefore be:

A Gram-negative coccus, usually occurring in pairs with flattened adjacent sides, which ferments dextrose and maltose with the production of acid, forms characteristic colonies, does not produce yellow pigment, usually grows at incubator temperatures, and is, as a rule, at some time agglutinable by polyvalent antimeningococcic serum.

Microörganisms having all of the above characteristics are certainly meningococci, but not all meningococci have each and every one of these characteristics.

The inclusion of agglutinability by polyvalent antimeningococcic serum in the definition of the meningococcus will be opposed by many on the very logical grounds that new serological groups, unrepresented in the serum, may be encountered; and also that strains fulfilling other requirements are considered meningococci whether they are agglutinated or not.

DISTRIBUTION

The normal habitat of the meningococcus is the naso-pharynx. In meningococcus infections it may be found in the cerebrospinal fluid, either intracellularly or extracellularly, in the blood, or in the petechiae of the skin. These are the usual sources from which the meningococcus is obtained.

DIAGNOSTIC PROCEDURES

Cerebrospinal fluid—This is usually obtained by lumbar puncture, the special needle for this purpose being inserted in the 3rd or 4th lumbar space. The size of the needle used depends to a certain extent upon the size of the patient, though sometimes a very thick and viscid fluid will make a large needle necessary in a child. An 18 gauge needle of 3 or 4 inches in length is the size in most common use, though 17 gauge may be necessary if the fluid is very thick, and 19 gauge is preferred by some. In small children, 20 and even 22 gauge of 2 inch length may be indicated. In cases where the spinal canal is blocked, cisternal puncture may be resorted to, and special needles are made for this purpose. This is not often necessary for an early diagnostic puncture.

The spinal fluid should be collected directly into several tubes, preferably at least three. This is especially important when the first fluid is blood tinged, for the presence of blood interferes with some of the examinations to be made. In some laboratories the rule is to use the second tube for cultures, and the third for cell counts, glucose determinations, and other chemical tests. Other individuals prefer the last tube for cultures. It is difficult to get an even suspension for counting if conical centrifuge tubes are used to collect the fluid.

The amount of fluid withdrawn, whether or not it is under increased pressure, and its appearance, *i.e.*, whether blood tinged or yellow, the degree of cloudiness, etc., should be recorded, and the fluid should then be sent immediately to a laboratory for examination. It should be kept warm, if possible, in this interval.

There are certain laboratory procedures which must be done immediately if they are to be of value. Chief among these is the cell count. The sample of

fluid should be free from blood, and the distribution of the cells should be even. Sometimes the fluid may be placed directly, without dilution, in a counting chamber, 9 large squares counted, and the result multiplied by 10/9 to obtain the number of cells found in 1 cu. mm. If the cells are too numerous to count by this method, the fluid may be diluted in a "white counting" pipette, as for a regular leucocyte count.

Special detailed directions for counting cells in cerebrospinal fluid may usually be found accompanying the counting chambers. The pamphlet prepared by the Arthur H. Thomas Company to accompany the Levy Chamber with the improved Neubauer ruling directs that the diluting fluid be drawn up to the mark "1" on the usual Thoma white cell pipette, and that the spinal fluid then be drawn up into the bulb and to the engraved mark above the bulb. After mixing, the first 2 or 3 drops are discarded, a subsequent drop placed in the counting chamber, and *5 minutes* allowed for the cells to settle. All cells in the entire ruling are counted and multiplied by 10/9 to ascertain the number of cells in 1 cu. mm. A special diluting fluid for cerebrospinal fluids is:

Methyl violet	0.2 gm.
Glacial acetic acid	10 c.c.
Distilled water	90 c.c.

This should be filtered to insure absence of artefacts.

Some workers feel that all spinal fluids should be diluted, even though only slightly turbid, to insure the absence of all red cells.

The practical value of a cell count is questioned by Dr. J. B. Neal[4] on the following grounds: it is difficult to obtain a uniform mixture of cells, not only because they settle out rapidly, but because the cell content varies among the several tubes collected; thus comparison of the count from day to day may be of no value or even misleading. Dr. Neal's opinion on this matter is also shared by others.

A differential count of the spinal fluid cells should also be made at this time. With very cloudy fluids smears for this purpose can be made without centrifugation; if the fluid is only slightly cloudy it may be centrifuged first and the sediment used. It must be remembered that leucocytes are fragile and that centrifugation hastens their destruction. Wright's stain, used as for blood smears, or May-Grünwald followed by Giemsa may be employed.

The May-Grünwald stain can be purchased by the pint ready for use. The method, as used by Dr. John Hays Bailey at the Municipal Contagious Disease Hospital in Chicago, is as follows:

1. Flood the slide with the stain, leave it on for 3 minutes, and then pour off. The slide may be washed with distilled water, but this is unnecessary. Do not blot. The preparation is not over-stained if left longer, *provided that it does not become dry.*
2. The diluted Giemsa is then poured on it (for 1–10 Giemsa, 10 minutes is average; for 1–15, 20 minutes is best. The methyl alcohol used for the Giemsa *must* be acetone free).
3. Wash thoroughly with distilled water from a wash bottle until the preparation has a pink color.

The differential count is a special aid in diagnosis with spinal fluids that are only slightly turbid. The predominance of polymorphonuclear leucocytes will aid in ruling out tuberculous meningitis, poliomyelitis, encephalitis, and lymphocytic choriomeningitis (aseptic meningitis, or serous meningitis).

Quantitative determinations of sugar in spinal fluid are done routinely in many laboratories, and are considered to be very important, not only as an aid to diagnosis, but in prognosis and in following the course of the infection.[4] Sugar determinations should be done at once and cannot be made in a blood-

tinged fluid. An adaptation of the method of Folin and Wu [5] to cerebrospinal fluids is found in the laboratory manual of Kolmer and Boerner,[6] and is quoted at the end of this report.

Direct precipitation tests upon the cerebrospinal fluid may often be of great value in arriving at an early diagnosis. For such a test to be successful it is important that the serum used should be rich in precipitins and that the spinal fluid should be obtained before serum therapy is begun.

Rake [7] has described such a test in which he has used monovalent rabbit sera especially high in specific group precipitins, and thus he has obtained information as to the "type" of meningococcus involved within a period of 2 hours instead of having to wait 2 or 3 days to ascertain this. He emphasizes the fact that negative results are apt to be obtained with spinal fluids in which the meningococci are few, thus causing very little specific substance to be present. His technic, which is also suitable for use with polyvalent sera, is essentially as follows:

One-tenth c.c. of serum is placed in a precipitation tube, and 0.1 c.c. of spinal fluid is layered over it. It is examined at once for a "ring" at the point of junction and incubated at 37° C. A reading is again made after 1 hour and then the tube is thoroughly shaken and replaced in the water-bath. Another reading for precipitate is made at 2 hours and the tubes are then placed in the icebox over night, at which time a final reading is made.

A stained smear of the centrifuged spinal fluid sediment is an important factor in early diagnosis. Dr, Scott, of the British Ministry of Health, reports that it is much easier to find meningococci in such smears if they are stained with watery, methylene blue or carbolthionin; and that smears so stained should always be looked at first, and a Gram-stained preparation examined after this. In some laboratories only the Gram-stained preparation is made. The flattened Gram-negative diplococci may be very abundant, both within and without the leucocytes; on the other hand, it may require careful search to detect them, and sometimes they cannot be found. A half hour is none too long to look for them. These stained smears should be made and examined as soon as possible.

Care should be taken not to jump at the conclusion that any Gram-negative bacteria seen are meningococci, for the Pfeiffer bacillus is very pleomorphic in spinal fluid and some Gram-positive cocci may seem Gram-negative in such smears. If Gram-negative diplococci are found they should be reported as such, and not called meningococci without further identification.

Further identification consists in cultivating the organism on a suitable medium, and examining its morphology in pure culture, its cultural characteristics, and its action upon dextrose and maltose, as well as its failure to ferment saccharose and levulose. The identity of the microörganism may be reported at this point. Corroboratory evidence can be obtained by agglutination with polyvalent serum, and this is especially valuable in the case of strains in which fermentative powers are not well developed. It must be remembered that some freshly isolated strains are inagglutinable. It is not absolutely necessary that the strain should agglutinate with polyvalent serum in order to be called a meningococcus.

The culture medium used for isolation of the meningococcus depends much on the laboratory or individual using it. Our own preference at the National Institute of Health is for 5 per cent rabbit's blood agar, made with a meat infusion base, and for a modification of the Hitchens' [8] simple semisolid 0.15 per cent agar medium made

from a beef infusion broth of pH = 7.4.* Some laboratories prefer adding 0.1–0.2 per cent dextrose to these media. Workers at the Rockefeller Institute use the following three media for isolation from spinal fluid, emphasizing that they must be both moist and warmed to 37° C.: (a) 4 per cent rabbit's blood "pneumococcus agar" plates, (b) semi-solid ascitic agar tubes, and (c) serum dextrose agar slants. They also favor planting two sets of these media and incubating one set under slightly increased CO_2 pressure. After the organism is isolated they prefer the rabbit's blood "pneumococcus agar" plate for identification.

For primary cultures the New York City Laboratories inoculate the following: red blood streak plate, glucose semi-solid stab, nutrient broth, coagulated blood agar slant, and a plain agar slant for control. They report that the largest number of positive cultures is obtained in the semi-solid medium.

The laboratories of the Municipal Contagious Disease Hospital in Chicago found a simple semi-solid agar medium so successful that they have discontinued the use of any other, using only it for isolation from spinal fluids.

The New York State Laboratories employ blood agar plates and a dextrose semi-solid medium, the plates being incubated in 5–10 per cent CO_2.

The British Ministry of Health reports inspissated egg as the favorite medium for isolation from spinal fluid, especially if the meningococci are scanty, or if the fluids have been sent from a distance, but prefer 10–20 per cent serum agar or 5 per cent blood agar for identification.

Thus we see that although there may be considerable variation in the details of technic in the isolation of meningococci from spinal fluid, the blood agar plate and the tube of semi-solid agar, with or without enrichment, remain the general favorites in practically all hands. All are agreed that a generous inoculum should be used; many insist that only centrifuged sediment should be used, though others use the uncentrifuged spinal fluid when the meningococci seem to be especially abundant in the stained smear.

The spinal fluid itself as a medium should not be ignored. Incubating it at 37° C. over night often results in a tremendous multiplication of the meningococci so that their detection in smears and their cultivation are made much easier. The New York State Laboratories incubate the sediment remaining after making primary cultures, and plate it daily on 3 successive days. The importance of the spinal fluid itself as a medium is recognized by many laboratories and is of special value in places where proper media are often hard to obtain. Cultures are often obtained from shipped fluids more than 24 hours old if large inocula are used. The British Ministry of Health recommends that specimens of spinal fluid that are to be shipped be mixed with an equal quantity of dextrose broth and incubated for 12 hours before being sent. Most workers try to keep the spinal fluids warm while they await examination, but Dr. Josephine Neal states that she gets her best results by keeping them in an icebox rather than in an incubator.

For fermentation reactions most laboratories seem to prefer a solid medium. A favorite is a 10 per cent ascitic fluid agar containing 1 per cent of the desired carbohydrate and phenol red, litmus, or andrade as indicator (medium 10, New York State and British Ministry of Health). Five per cent serum is added by some instead of the ascitic fluid, and the Rockefeller Institute adds 5 per cent hemolyzed blood to a beef heart agar base.

* Formulae for these media and for others to be described will be found at the end of this report.

Not all laboratories prefer solid media. The Municipal Contagious Disease Hospital laboratory in Chicago uses the ordinary semi-solid agar medium with carbohydrate and indicator added. At the National Institute of Health in Washington, we prefer a simple semi-solid medium made of peptone; 0.15 per cent agar, salts, and carbohydrate, with brom-thymol-blue added—a medium devised by Enlows.[9] This is not a rich medium, and for initial cultures from spinal fluid we have added carbohydrate and indicator to the usual hormone semi-solid media (medium 11). If such cultures are made directly from spinal fluid, it is frequently possible to finish the complete identification of the meningococcus in less than twenty-four hours. Most meningococci grow nicely on the Enlows medium, but especially delicate strains are placed on serum-carbohydrate-agar (medium 10) slants with added indicator (brom-thymol-blue).

For maintenance of cultures of meningococci there are three types of media in favor: (a) semi-solid agar, with or without ascitic fluid and with various modifications, (b) serum dextrose agar slants, and (c) egg slants.

(a) In a simple 0.15 per cent agar medium, with a meat infusion base, cultures of meningococci will usually need to be transplanted about every 2 weeks, though some may live much longer. At the National Institute of Health we have adopted Murray's[10] suggestion that small amounts of KCl and $CaCl_2$ be added. Cultures upon this modified medium are transplanted about once a month (medium 3). With the addition of ascitic fluid (medium 5) this period between transplants may be lengthened to 2 months (New York State Laboratories). There is no doubt in our minds that Murray's EDB[11] medium prolongs the virulence of meningococci, though it does not maintain this quality permanently. The elaborateness of preparation and titration of this medium has prevented its becoming as popular as it deserves to be.

(b) Serum dextrose agar (medium 6) is an excellent medium for meningococci, its drawback being that such cultures are very short lived. Some laboratories transplant them every 48–72 hours, whereas others find once a week sufficiently often. Even so, many laboratories prefer to maintain their most important strains on this medium, feeling that the antigenic and immunizing properties of the strains are preserved best on it. There is very little data on the effect of frequent transplanting required by this medium upon the virulence of the strains.

(c) For longevity of cultures egg slants seem to hold first place. Such cultures, sealed, usually remain viable at 37° C. for over a year. In transplanting such cultures it is important to transfer them first to dextrose serum agar, blood agar, or egg slants, and then 48 hours later put them again on the egg slants on which they are to be stored. There seems to be considerable difference of opinion as to the effect of this medium upon virulence, antigenicity, and serological relationships.

(d) With all of the above media it is understood that incubation and storage are at 35–37° C. Although room temperatures and ordinary icebox temperatures do not permit long viability of meningococci, storage in a frozen state at —15° C. has been found to maintain glycerine covered cultures or glycerine suspensions for more than 2 years.[12] Virulence is also prolonged to some extent by this method of storage.

When agglutination is to be done to confirm the identity of the meningococcus, growth, not more than 24 hours old, from solid media is used. The favorite medium for this purpose is a 0.5 per cent dextrose agar. Growth is

suspended in 0.85 per cent sodium chloride solution of a known pH, and the suspension diluted to a density equivalent to 500 p.p.m. of the silica standards described in *Standard Methods of Water Analysis*.[13] Such a suspension contains, on an average, about 1 billion organisms per c.c., though individual suspensions vary greatly, as do individual strains.

It is important to know the pH of the salt solution used, for a culture that may be inagglutinable at pH = 7.8 may agglutinate nicely at pH = 6.8. Serum dilutions of 1–25 to 1–1,600 or higher should be made, making the final dilutions after the suspension is added 1–50 to 1–3,200. The total amount used in an ordinary sized agglutination tube is 1 c.c., though some prefer smaller tubes and lesser quantities. Normal horse serum in dilutions of 1–25 and 1–50 and a saline control should also be used. This whole test is incubated at 55° C. for 18 hours and then read. Some workers prefer incubation at 37° C. for 2 hours and storage in an icebox over night before reading.[14]

Some laboratories use microscopic slide agglutination. The greatest advantage of this procedure is that individual colonies from a primary culture may be used and a correct diagnosis arrived at quickly. The usual method for performing these is as follows [15, 16]:

Polyvalent antimeningococcic horse serum and normal horse serum are diluted 1–10 with physiological salt solution, and a loopful of each placed on a slide. Some of the suspected colony is emulsified in each, or, some of the colony is emulsified in the normal serum and some of the suspension transferred to the drop of diluted immune serum.

Agglutination may be observed macroscopically and microscopically. Organisms of characteristic morphology and staining, which are agglutinated only by the immune serum, may be tentatively considered meningococci. This method is not used for "typing."

Whether or not it is of value to "type" cultures of meningococci as a routine procedure is still an open question. As an aid in the diagnosis of meningococcus meningitis, and as a help in treatment of the individual patient, its value is uncertain at the present time, since serum used in treatment is entirely polyvalent in this country. Should the technic of preliminary "typing" by a precipitation reaction between spinal fluid and monovalent serum come into general use, it should theoretically be possible to use monovalent serum for treatment. Under these circumstances it would be important to type the meningococcus obtained from the spinal fluid as a confirmation of the precipitin test.

The British have already put these principles into use. Besides the usual polyvalent serum, they use a monovalent group I serum (including "types" I and III) for group I–III infections. For this reason the British Ministry of Health recommends routine "typing" as a guide in serum therapy as well as an index of changes in the serological type prevailing in different areas of the country. They often make use of a precipitin test upon centrifugal spinal fluid to make an early determination of the type, and confirm this later by agglutination of the culture.

Although unnecessary for diagnosis and treatment in the present state of our knowledge and experience, "typing" is of great serological and epidemiological interest, and is well worth doing from these standpoints if the laboratory has time and sera that are sufficiently specific. Information about the "types" or "groups" that are prevalent in a community or in a given epidemic can only be determined in this way.

"If "typing" is to be done, it is essential that the strains of meningococci with which the rabbits are to be immunized are chosen with great care. The serological groups are not clear-cut and almost every degree of "overlapping" can be found. The strains chosen should correspond as nearly as possible to the original type strains described by Gordon and Murray.

A satisfactory method of immunizing rabbits is by 3 courses of intravenous injections of freshly made suspension of young cultures of meningococci on 3 successive days, these series being 1 week apart. A week after the third series a trial bleeding is made, and if the serum is satisfactory the animal is bled from the heart. If the immunization extends over too long a period, the titer of the serum may be increased, but this is apt to be at the expense of specificity and more "cross agglutination" between groups is apt to result.

When strains are being "typed" it is best to include in the test the specific strains with which the sera were made and also a polyvalent horse serum of good agglutinating titer. The British Ministry of Health gets best "typing" results with the I–III group by using absorbed sera.

Blood—It is becoming more and more common to make blood cultures as an aid to diagnosis in meningococcus infections. Especially are these of value in very early cases, and in those without meningeal symptoms. These should be made as soon after the blood is drawn as possible—preferably at the bedside. Small flasks of hormone broth may be used (medium 1), *i.e.*, about 10 c.c. of blood added to 100 c.c. of warmed broth in a 250 c.c. flask. This gives a relatively large surface, which is desirable, as the meningococcus tends to grow at the surface. Semi-solid agar (medium 3) gives good results, as meningococci do not grow easily in liquid media, as a rule. Some people add the blood to hormone agar (medium 2), melted and cooled to 45° C., mix, and pour into Petri dishes. Addition of 0.1–0.2 per cent dextrose to these media may enhance the growth. When initial cultures are obtained, identification of the organism proceeds as usual.

It is with cultures isolated from blood that differentiation from the gonococcus is most often important, though this situation may also arise with cultures from spinal fluid. Definite rules for such differentiation are not easy to set down. The gonococcus is much more exacting in its growth requirements, and shows very small fine colonies on solid media. It ferments only dextrose. It is, as a rule, well agglutinated by polyvalent antimeningococcic serum, and by many of the monovalent sera, especially those of group II, so that agglutination does not make a satisfactory basis for differentiation. Some people who have worked much with gonococci feel that complement-fixation is more reliable.

Petechiae — Although meningococci are present in the petechiae, isolation from them is seldom attempted during life. Occasionally, after death, this procedure allows a diagnosis when other methods are unsuccessful, especially in fulminating cases which have died without showing meningeal symptoms and from which no blood cultures were made. The microörganisms may be seen in properly stained sections (Gram), and they may often be cultivated if the tiny bit of skin is placed in semi-solid media.

Nasopharynx—Very few hospitals and laboratories make nasopharyngeal cultures from cases of meningitis routinely. So it is difficult to know how constantly and for how long the meningococcus is found there. It can be said, however, that those who have looked for it there have frequently found it. Most often nasopharyngeal cultures are made for the purpose of

detecting carriers. In any case, the technic is the same, and is described in great detail in many manuals.

Many people like to use a West tube in making these cultures, as the swab is protected from contamination with the throat flora. Personally I much prefer a simple bent wire swab and an ordinary wooden tongue depressor. These wire swabs have been used extensively by the Army, Navy, and the U. S. Public Health Service, and are described in the pamphlet, *Standard Technique of Meningococcus Carrier Detection*, compiled jointly by them some years ago.[17] The swab is a naked flexible wire about 25 cm. long, with one end well covered by an absorbent cotton pledget, and the other end bent into a loop for a handle. The last 1–2 cm. of the cotton covered end is bent to an angle of about 30–40°.

A straight wooden swab stick is used by some, but it is less easy to obtain satisfactory material with.

In any case the swab should be passed behind the uvula and should be moved gently to and fro over the posterior and upper pharyngeal wall. It should not touch any part of the mouth or throat. A good light is important. In a search for carriers, the subject should sit facing the light. In the case of patients who are ill, a flash or head light may be used.

The blood agar plate is the medium of choice for the isolation of the meningococcus from the nasopharynx. A hormone agar with 5 per cent defibrinated rabbit's blood (medium 2) is our own choice. Dr. Rake, whose recent work with carriers has been intensive, prefers adding 4 per cent rabbits' blood to "pneumococcus agar" (medium 4). For a time laked blood agar plates were used extensively, and some people still prefer them. It has the advantage of making the meningococcus colony appear more translucent, but the disadvantage of not showing the zones of hemolysis or methemoglobin produced by many of the bacteria from which the meningococcus is to be differentiated.

In the usual blood agar plate the translucent meningococcus colonies are easily distinguished from those of the commoner inhabitants of the nasopharynx, except for the other members of the genus *Neisseria*, which are often confused with those of the meningococcus. It must not be forgotten that occasionally meningococcus colonies may be very large.

Differentiation of the meningococcus from other *Neisseria* often requires careful cultural, biochemical, and serological studies for its sure identification. This makes carrier detection a laborious procedure.

Sometimes the proportion of meningococci to other bacteria in the nasopharynx will be very small; at other times it may be found in practically pure culture.

The suspected colonies are ringed and a Gram-stained smear made from a part of each. If this smear shows Gram-negative cocci of typical morphology, the colony is picked to a blood agar or serum dextrose agar slant and incubated at 37° C. The next day cultures are made to test its fermentation reactions, it is transferred to semisolid agar to keep alive for future use, and the remainder of the 18–24 hour growth emulsified in salt solution to use for agglutination with both polyvalent and type sera. If slide agglutination is used at this stage, it should be confirmed later by the test tube method.

It is not necessary to "type" each culture for mere carrier detection, and when such a survey is being made on a large scale it is sometimes advisable, as well as necessary, to omit the "typing." But from an epidemiological point of view, "typing" is very important in carrier surveys, since it indicates the nature of the strains preva-

lent, and often also the trend of an epidemic. For this reason, it is often desirable to "type" the recovered meningococci later if there is no opportunity at the actual time of isolation. "Typing" a strain immediately after its isolation frequently gives a much better idea of its antigenic pattern, since some strains have a tendency to "spread" serologically on prolonged laboratory maintenance, so that it is more difficult later to determine its grouping accurately or its relation to the other strains in a given epidemic.

Inagglutinable strains may be encountered. If these are not true meningococci they will be apt to be agglutinated to a slight extent by normal horse serum, or they may ultimately form a yellow pigment or have peculiar fermentation reactions. If a strain is not agglutinated by normal horse serum, does not form pigment, and ferments dextrose and maltose only, it is fairly safe to consider it a meningococcus. If, however, it fails to ferment one or both of these sugars, as some strains do immediately after isolation, it is important to get serological confirmation of its identity. Sometimes a slight lowering of the pH of the salt solution used in the suspension and serum dilutions will turn the trick (in such a case controls without serum should show that it is not merely an acid agglutination). Sometimes, if a strain is plated out and several colonies picked and studied, one colony will be found to agglutinate. And sometimes an indirect method must be resorted to—a rabbit is immunized with the strain in question and the resulting serum used to agglutinate known meningococci. This last step is seldom necessary in order to get agglutination with a good polyvalent serum, but is not infrequently resorted to in "typing." Usually strains of meningococci that are peculiar in these ways become quite typical in agglutination and fermentation after a more or less long period of laboratory maintenance.

FORMULAE FOR CULTURE MEDIA

1. Beef Infusion Bouillon (National Institute of Health)

(Used as a base in making semi-solid media for stock culture maintenance, and for other media)

Constituents—

1. Beef steak (freed of fat and bone)	500 gm.
2. Peptone (1.0%)	10 gm.
3. Sodium chloride, C.P. (0.5%)	5 gm.
4. Distilled water	1,000 c.c.

Preparation—

1. Remove all fat, bone, and tendons from beef steak and chop into small pieces.
2. Add distilled water, mix well, and allow to infuse in ice chest for not less than 18 and not more than 24 hours.
3. Remove from ice chest, heat in streaming steam (100° C.) for 1 hour, then under pressure of 15 lb. for 30 minutes.
4. Squeeze through cheese cloth until 1,000 c.c. is obtained. If unable to obtain this amount, restore volume with distilled water.
5. to this broth add:

Peptone	10 gm.
Sodium chloride, C.P.	5 gm.

Mix well, stir until ingredients are dissolved.

6. Adjust reaction, using NaOH solution, to pH 7.6 or desired reaction.
7. Heat in streaming steam (100° C.) for 30 minutes.
8. Determine reaction and, if necessary, adjust to 0.2 points above desired final pH to compensate for the acid drift encountered in sterilization.
9. Filter through moist filter paper.
10. Distribute into chemically clean containers.
11. Sterilize—Steam autoclave, 15 minutes 15 lb. pressure.
12. Incubate for not less than 12 hours at a temperature of 37° C. for a check as to the efficiency of the sterlization procedure.

NOTE: Heavy precipitates of phosphates are occasionally thrown down at time of sterilization, thereby making it necessary to repeat the process of filtration and sterilization; that is, if a clear broth is desired immediately. However, if not needed for immediate use and the medium is allowed to stand for a number of weeks, the phos-

phate precipitates will dissolve, eliminating the process of filtration which extracts valuable nutritive materials.

The prolonged heating period (1 hour at 100° C. and 30 minutes at 15 lb. pressure, Step No. 3) applied to the cooking of the beef infusion, tends to stabilize the corrected reaction of the finished medium.

The color of the finished broth will vary due to the amount of blood pigment in the beef steak used. Prolonged heating and sterilization periods will also affect the color of the finished product.

Various infusion bouillons may be prepared by substituting veal, heart, liver, brain, etc., in place of the beef steak in the above formula and following precisely the same preparation procedure given for the Beef Infusion Bouillon.

2. Nutrient Agar Agar (National Institute of Health, Washington)

(Meat Infusion Agar)

(Used as base in making blood agar plates, semi-solid agar medium for serum-carbohydrate slants for fermentation, and for dextrose agar for shipping cultures)

Round steak, free from fat, chopped fine	500 gm.
Tap water	1,000 c.c.

Mix and let stand in icebox for not less than 18 hours and not more than 24 hours.

Heat in streaming steam (100° C.) for 1 hour and under pressure of 15 lb. for 30 minutes.

Squeeze through cheese cloth until 1,000 c.c. is obtained. If unable to obtain this amount make up loss with water.

To this broth add:

Peptone	10 gm.
Sodium chloride C.P.	5 gm.

Adjust reaction, using NaOH solution, to pH 8.0.

Heat in streaming steam for 30 minutes.

To this broth add:

Shredded * agar-agar	20 gm.

Stir thoroughly.

Heat in streaming steam for 1 hour and under pressure of 15 lb. for 30 minutes.

At this point turn out flame under autoclave. Do not open pet cocks. This will allow pressure to drop slowly. Do not open sterilizer for at least 18 hours. This will allow agar to solidify and all precipitates which have dropped to bottom of container may be cut off when the agar is slipped from the container.

The clear portion is melted and, after adjusting to pH 7.6, is filled into the desired containers and sterilized.

Sterilization: Autoclave 15 minutes 15 lb.

For blood agar plates, melt the above nutrient agar. Cool to between 42 and 45° C., add 5 per cent defibrinated rabbit's blood, mix and pour. Horse or sheep blood may be used instead of rabbit.

For semi-solid agar, see formula 3.

For serum carbohydrate slants see formula 10.

For dextrose agar slants, 1 per cent or 0.5 per cent dextrose is added to this medium. With this medium the carbohydrate may be added directly, or, even better, a 20–50 per cent solution, sterilized by heat, may be kept on hand, and enough added before dispensing to make the desired concentration.

3. Semi-solid Agar Medium for Meningococci (National Institute of Health)

(A modification of Hitchens's Medium)

Constituents—

1. Beef infusion bouillon (pH 7.4)	2,700 c.c.
2. Nutrient agar-agar	300 c.c.
3. Potassium chloride, KCl C.P.	0.6 gm.
4. Calcium chloride, $CaCl_2$ C.P.	0.3 gm.

Preparation—

1. Melt agar and heat bouillon to 100° C.
2. Mix agar and broth well.
3. Add potassium chloride and calcium chloride.
4. Stir until thoroughly dissolved.
5. Determine reaction and, if necessary, correct to pH 7.3.
6. Dispense into tubes.
7. Sterilize—15 minutes, 15 lb. pressure.
8. Incubate for not less than 12 hours at a temperature of 37° C. for a check as to the efficiency of the sterilization procedure.

* Powdered agar-agar may be used in place of the shredded agar-agar, but the following steps are required: sprinkle the powdered agar-agar on the surface of the bouillon; let stand until the agar-agar has dropped to the bottom of the container; stir briskly; then heat.

4. Pneumococcus Agar (Rockefeller Institute)

Fresh beef heart (free from fat and gristle)	500 gm.
Witte peptone	10.0 gm.
Sodium chloride	5.0 gm.
Agar	20.0 gm.
Dextrose	0.3 gm.
Tap water	1,000.0 c.c.

For meningococci, 100 c.c. of citrated rabbits' blood are added to each 250 c.c. of medium, and plates poured.

5. Semi-solid Ascitic Agar (Rockefeller Institute)

Heart muscle (free from gristle)	1 lb.
Distilled water	1,000 c.c.
Peptone (Witte's)	10 gm.
NaCl	5 gm.
1 per cent agar	0.25 gm.

Chop meat and infuse in water over night in refrigerator. Heat infusion to 85° C. for 30 minutes. Filter through Prat-Dumas filter paper No. 50 and make up to original amount with distilled water. Add peptone and NaCl and bring to the boil. At boiling point adjust pH to 7.8 and add agar. Heat in Arnold for 1 to 1½ hours. Filter through absorbent cotton into flasks and sterilize at 116° to 118° C. for 20 minutes in the autoclave. The final pH should be 7.6 to 7.4.

To this agar, when melted and cooling, add an equal amount of sterile ascitic fluid.

6. Glucose Semi-solid Medium (New York City Department of Health)

Nutrient veal infusion broth	1,000 c.c.
Dextrose	1%
Agar	0.25%

Adjust reaction to pH 7.2–7.4. Tube and sterilize in Arnold apparatus for 1 hour on 2 successive days. This medium should be firm enough to hold its shape when gently inverted, should offer no resistance to the needle, and should be semi-fluid when at incubator temperature.

7. Serum Dextrose Agar Slants (Rockefeller Institute)

To 400 c.c. of stock 2 per cent agar add 80 c.c. of sheep serum water; sterilize in Arnold for ½ hour. Cool slightly and add 1 per cent dextrose.

Sheep serum water—1 part serum to 3 parts water which is sterilized in the Arnold for 3 consecutive days for 30 minutes. It is resterilized just before adding.

Dextrose solution—A 50 per cent solution sterilized in the Arnold for 2 consecutive days for 30 minutes.

8. Solidified Egg Medium (Modified Dorsett's Egg Medium) (National Institute of Health)

Constituents—

1. Fresh eggs	4 eggs
2. NaCl solution (0.85% solution)	25 c.c.

Preparation—

1. Scrub eggs with brush in soap and water. Allow eggs to dry.
2. Place eggs in wire basket and dip into 95 per cent alcohol. Allow to remain a few seconds, after which time it is withdrawn and the small amount of alcohol which still remains on the basket and eggs is ignited in order to remove the alcohol and help sterilize the shells.
3. The shells are then broken using aseptic technic and the whites and yolks placed in a sterile container.
4. Add 25 c.c. of sterile 0.85 per cent NaCl solution.
5. Mix well with sterile beater.
6. Filter through sterile cheese cloth.
7. Dispense into sterile tubes.
8. Slant in an inspissator, heat very slowly until solidified. Do not allow heat to rise above 76° C.
9. Cool and incubate.

9. "Chocolate" Coagulated Blood Agar (National Institute of Health)

Constituents—

1. Sterilize veal Infusion agar (see note below)	100 c.c.
2. Fresh, sterile, defibrinated, sheep blood	10 c.c.

Preparation—

1. Melt agar (keep sterile).
2. Cool agar to 45° C.
3. Add sterile sheep blood and mix well.
4. Heat slowly in Arnold sterilizer or in a water-bath. Allow temperature to rise very slowly until medium has a very definite chocolate color. Excessive heating will cause blood to coagulate and the finished medium will show clumps of cooked blood instead of the uniform, smooth, chocolate color desired.

5. Dispense into sterile containers and allow to cool.

6. Incubate for not less than 12 hours at a temperature 37° C. for a check as to the efficiency of the sterilization procedure.

NOTE: Veal infusion agar is prepared according to the directions given for beef infusion agar substituting the same amount of veal in place of the round steak. All other ingredients remain the same and the preparation steps are to be followed exactly as listed.

10. Solid Medium for Fermentation Tests (New York State Laboratories and National Institute of Health)

Sugar-free beef-infusion agar, 2% with indicator	200 c.c.
Carbohydrate 10.0% solution	1% or 20 c.c.
Ascitic fluid	10% or 20 c.c.

Procedure—

Melt the agar and cool to 50° C.

Add carbohydrate and ascitic fluid (both previously warmed to 50° C.) .

Mix well and dispense.

Slant tubes immediately to solidify.

Incubate for 48 hours at 37° followed by 48 hours at room temperature.

Examine for contaminants.

The carbohydrate solution has been sterilized by filtrations through a sterile Berkefeld candle.

In some laboratories the serum or ascitic fluid is omitted.

11. Semi-solid Media for Fermentation Tests (National Institute of Health)

(This is usually medium 3 with indicator and 5 per cent of a filtered 20 per cent solution of the desired carbohydrate added.)

12. Enlows's Sugar Free Medium (Semi-solid) for Fermentation Tests

Peptone	5.0 gm.
Na_2HPO_4 (sodium phosphate)	7.2 gm.
KH_2PO_4 (potassium phosphate)	0.7 gm.
Agar-agar	2.0 gm.
Tap water	900.0 c.c.

The above substances are mixed well and heated for 30 minutes at 100° C. (streaming steam) and then given 15 lb. pressure for 15 minutes in the autoclave.

Filter through moist filter paper while hot. Keep solution as hot as possible during filtration process.

Three drops of concentrated brom-thymol-blue solution is added to every 500 c.c. of the clear filtered solution. The color corresponds to pH = 7.6 in the brom-thymol-blue color range.

Pour into suitable containers and sterilize 15 minutes at 15 lb. This is the basic medium.

Carbohydrate solutions are prepared as follows:

Distilled water	100 c.c.
Carbohydrate	4.5 gm.

Mix thoroughly and sterilize by filtering through a sterile Berkefeld filter.

The above solution (100 c.c.) is added to 900 c.c. of the sterile basic medium and mixed.

Dispense into sterile tubes.

Incubate 48 hours to check sterility.

13. Preparation of Concentrated Brom-Thymol-Blue Indicator (National Institute of Health)

Brom-thymol-blue (dibromo-thymol sulphonphthalein) finely pulverized	1.0 gm.
N/20 NaOH	32.0 c.c.

Grind together in a mortar until in solution.

QUANTITATIVE ESTIMATION OF SUGAR IN CEREBROSPINAL FLUID

(Kolmer and Boerner. *Approved Laboratory Technique*, 1931, p. 242)

Principle—

Proteins present are precipitated by tungstic acid and determination is carried out by the Folin-Wu method, using 1–5 dilution of filtrate.

PROCEDURE

1. With a 1 c.c. pipette transfer 1 c.c. of spinal fluid to a *clean, dry* test tube.

2. Add with a pipette 3 c.c. distilled water.

3. Using a graduated 1 c.c. pipette, add 0.5 c.c. of 10 per cent sodium tungstate.

4. Add 0.5 c.c. of ⅔ normal H_2SO_4.

5. Mix well and let stand 5 to 10 minutes.

6. Filter.

7. Pipette 2 c.c. of the clear filtrate into a Folin-Wu sugar tube and proceed as with blood. See *"Blood"* below.

8. Calculation, using standard 1:

$$\frac{20}{R} \times 50 = \text{mg. dextrose per 100 c.c. fluid.}$$

R = reading.

9. Pipette and test tubes should be abso-

lutely clean and dry or the error will be large.

10. If the cerebrospinal fluid contains a little blood, centrifuge and use the clear supernatant fluid.

For Blood—

1. Pipette 2 c.c. of tungstic acid blood filtrate into a special Folin sugar tube.
2. To a similar tube, add 2 c.c. of standard sugar solution B, and to a third add 2 c.c. of standard sugar solution C, containing 20 mg. dextrose per 100 c.c.
3. To each tube add 2 c.c. of the molybdate phosphate solution.
4. Transfer the tubes to a boiling water-bath and heat for 6 minutes.
5. Cool for 2 to 3 minutes in a cold water-bath without shaking.
6. Add to each tube 2 c.c. of the molybdate phosphate solution.
7. Let stand for 3 minutes; dilute the resulting solution to the 25 c.c. mark.
8. Insert a rubber stopper and mix.
9. Compare in a colorimeter with the nearest matching standard set at 20.

The two standards are adequate for practically all cases as they cover a range from about 70 to nearly 400 mg. of dextrose per 100 c.c. of fluid tested.

REAGENTS FOR BLOOD SUGAR DETERMINATIONS

Alkaline copper tartrate solution:

Dissolve 40 gm. of pure anhydrous sodium carbonate in about 400 c.c. of distilled water in a liter flask. Add 7.5 gm. of tartaric acid, and when this is dissolved add 4.5 gm. of crystallized copper sulphate. Mix and make up to a volume of 1 liter.

Test for the absence of cuprous copper by transferring 2 c.c. to a test tube and adding 2 c.c. of the molybdate phosphate solution; the deep blue of the copper should almost completely vanish.

If the chemicals used are not pure, a sediment of cuprous oxide may form in the course of 1 or 2 weeks. If this happens, remove the clear supernatant reagent, or filter through a good quality filter paper. Test for copper. This reagent keeps indefinitely.

Molybdate-phosphate solution:

Transfer 70 gm. of molybdic acid (C. P. Bakers analyzed "special") to a liter beaker. Add 10 gm. of sodium tungstate, 400 c.c. of 10 per cent sodium hydroxide, and 400 c.c. of distilled water. Boil vigorously for 20 to 40 minutes (to remove ammonia present in molybdic acid). Cool, and dilute to about 700 c.c. Add 250 c.c. of concentrated (85 per cent) phosphoric acid. Dilute to 1,000 c.c.

The solution should be water clear. If it has a yellow tinge the chemicals used are not pure enough and there will be an error in the determination.

Benzoic acid:

Dissolve 2.5 gm. benzoic acid in 1 liter of hot water and cool. Transfer to a bottle; the solution will keep indefinitely. Filter as necessary.

Standard sugar solutions:

A. Stock—Weigh 1 gm. of pure dextrose (glucose) on an analytical balance and dissolve in about 50 c.c. of the benzoic acid solution. This 1 per cent standard stock solution keeps indefinitely.

B. Standard containing 10 mg. dextrose per 100 c.c.—Pipette 5 c.c. of stock solution into a 500 c.c. volumetric flask and dilute to the mark with benzoic acid solution.

C. Standard C containing 20 mg. dextrose per 100 c.c.—Pipette 10 c.c. of stock solution into a 500 c.c. volumetric flask and dilute to the mark with benzoic acid solution.

Diluted standards B and C should be made fresh every month.

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ACKNOWLEDGMENTS: For valuable information, suggestions, and criticisms, I am especially indebted to Dr. Josephine B. Neal of the New York City Health Department; to Dr. Geoffrey W. Rake of the Rockefeller Institute; and to Dr. W. M. Scott of the British Ministry of Health, London. I am grateful to Dr. John Hays Bailey and to Lucy Retondo of the Municipal Contagious Disease Hospital in Chicago for many practical laboratory suggestions.

SARA E. BRANHAM, *Referee*

Serological Tests for the Diagnosis of Syphilis*

THE trends indicated by the volume of specimens received for examination suggest that each year greater reliance is placed by physicians and health officials on the results of serological tests as an aid in the diagnosis and as a guide for the treatment of syphilis. The value of the procedures at present in general use in North America is thus emphasized. This situation, however, should not inhibit efforts toward further improvement. A still greater sensitivity is probably desirable; also simplification of procedures would, without doubt, result in a much wider application of such tests.

The data obtained in the study of serological tests for syphilis conducted under the auspices of the U. S. Public Health Service [1] are in general accord with the results of the investigation of the problem undertaken for the Committee on Standard Methods of the Laboratory Section of the American Public Health Association.[2] In both instances, the findings suggest that the complement-fixation test can, with safety, be made somewhat more sensitive than precipitation tests; also that these two types of procedures as developed in the United States and Canada differ in relative efficiency from those represented at the conferences at Copenhagen [3] and Montevideo,[4] sponsored by the League of Nations, where the results indicated that the complement-fixation tests were less satisfactory than some of the precipitation tests with which they were compared. The reactions reported by Dr. Kahn, who participated in all of the studies, furnish an excellent basis for comparative analysis.

The findings with specimens from lepers secured in the recently completed investigation were of special interest. A thorough study of the behavior of sera from patients with this disease is indicated. The high incidence of yaws or syphilis in some of the countries where leprosy is prevalent complicates the problem.

The desirability of employing a presumptive technic for the selection of specimens which would require further study was suggested to your committee last year. A similar recommendation was included in the report of the committee acting for the U. S. Public Health Service.[1] Such a procedure would not only facilitate the work, but also encourage a more careful study of the specimens selected for evaluation. A presumptive complement-fixation procedure with a sensitive cholesterolized antigen, less than two units of complement, and fixation over a prolonged period at from 3 to 6° C., followed by incubation at 37° C., should provide an adequate means for detecting specimens which would fail to react in completely controlled tests with such antigens. The use of a relatively small amount of complement should result in reactions being indicated even with specimens which give more marked fixation with small rather

* Report of the Referee on Methods for the Serological Diagnosis of Syphilis for the Standard Methods Committee on Diagnostic Procedures and Reagents.

than large amounts of serum. With the majority of specimens from patients with syphilis, more sensitive reactions have been obtained with cholesterolized antigens than with non-cholesterolized. Specimens which react with the non-cholesterolized antigen only are encountered so rarely that the adoption of a procedure for their detection would seem unnecessary. In a presumptive procedure, controls for anticomplementary properties would not be required since any specimen with which evidence of a significant degree of fixation occurred would be carefully tested.

The use of an oversensitive precipitation test should, in a similar manner, permit the selection of sera that would fail to react in more delicately adjusted tests of this type.

In many laboratories, entirely negative findings are obtained with at least 75 per cent of the specimens received, and 25 per cent of them, or less, either give reactions of varying degrees or are anticomplementary, or for some other reason are not satisfactory for the test. A presumptive procedure should, of course, be oversensitive, but should be so adjusted that under the conditions just mentioned approximately 70 per cent could be promptly excluded as failing to react. The remaining 30 per cent could then be studied fully. The saving of time and materials that would result might be illustrated by a concrete example. With 400 specimens per day, preliminary complement-fixation and precipitation procedures which would employ one tube each (without controls) would require 800 tubes. If negative findings were obtained with 70 per cent of the specimens, reports could be sent promptly on 280 of them. Assuming 8 tubes would be used for fully controlled complement-fixation and precipitation tests on the remaining 120 specimens, 960 additional tubes would be needed or a total of 1,760 tubes, while if a complete test with 8 tubes had been employed on the original 400 specimens, 3,200 tubes would have been required. As an increasingly large proportion of specimens is collected from persons undergoing physical examinations, those admitted to hospitals, and similar groups where the percentage of reacting sera is found to be small, the greater will be the saving in time and materials if a presumptive method is used.

Having the preliminary procedures as well as the final tests handled independently by two groups of workers would furnish protection against technical inaccuracies.

The use of presumptive tests may be inhibited through a fear of obtaining oversensitive results. Preliminary procedures without controls would not have this disadvantage, since diagnostic significance would be attached only to negative findings secured by this means.

By reducing the work with specimens which fail to react, quantitative procedures might be employed with some of the other sera, especially those which give partial or atypical reactions; for example, those which have been found to give reactions with one antigen only or at one temperature only. Careful studies of such specimens might be helpful, particularly in instances where the patients are not found to have a definite history or definite clinical manifestations of syphilis. A means for the evaluation of the relative specificity of the fixation obtained would be especially desirable in such cases. The procedure described by Wadsworth, Maltaner, and Maltaner,[5, 6] provided the technic could be made applicable for complement-fixation tests for syphilis in which small amounts of serum are used, may furnish a method for determining the significance of reactions of the type mentioned, as well as those secured with specimens from lepers and the ones occasionally ob-

tained with blood from patients having malaria, tuberculosis, and other febrile diseases.

Preliminary findings indicate that presumptive procedures of the type outlined would markedly facilitate the work of a routine nature without jeopardizing accuracy. Also, the comparatively small percentage of specimens with which partial reactions are obtained should permit them to be studied fully, thus facilitating the wider use of the test, in view of the greater economy, and also providing additional information in regard to reactions of the type which, under present conditions, sometimes offer particular difficulties of interpretation.

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RUTH GILBERT, *Referee*

Tentative Methods for the Cultivation and Isolation of Tubercle Bacilli and the Production of Tuberculin*

I. General statement
II. Preparation of specimen for examination
III. Preparation of reagents
IV. Preparation of culture media
V. Production of tuberculin (Koch's A.T.)

Cultural Method for the Primary Isolation of M. Tuberculosis

The cultural method for the isolation of *M. tuberculosis* has now gained wide recognition. It has been found to be quite as reliable in most instances as the method of guinea pig inoculation and has the advantage of being more economical and time saving. Guinea pig inoculation should be carried out on specimens of urine.

Method of Procedure—One of the most important steps in the preparation of routine specimens for the culture of tubercle bacilli is the method of treating the specimen in order to eliminate the contaminating organisms, which would hinder or obscure the growth of the tubercle bacillus. It is also essential that whatever form of treatment is instituted, it should not kill any tubercle bacilli present in the specimen.

1. The specimen as received is placed in a sterile 6" test tube, care being taken to deliver it to the bottom of the tube. This is done to prevent, as far as possible, contaminating the side of the tube. The tube is well flamed and a sterile cotton plug is inserted.

2. Equal parts of 3 per cent hydrochloric acid containing bromcresol purple is added to the specimen. Should there only be a sedimented specimen, 5 c.c. of this reagent is added. This acid solution is 3 per cent of the concentrated hydrochloric acid, and is not 3 per cent hydrogen chloride.

3. After the addition of the 3 per cent hydrochloric acid solution to the specimen, the tube is shaken by a side to side motion and the upper portion of the tube is well flamed to within an inch of the upper level of the liquid. A fresh sterile cotton plug is inserted in the tube.

4. The partially treated specimen is left at room temperature for 2 hours, after which time it is neutralized by the addition of 3 per cent sodium hydroxide to pH 7.0. This reagent is added aseptically to the tube.

5. A rubber cap is placed over the tube, in order that the overlapping cotton plug will be held in place. The neutralized specimens are then centrifuged at high speed for ½ hour.

6. After centrifugalization, the supernatant fluid, to within ½" from the top of the sediment, is withdrawn by means of a suction apparatus, which is attached to a rubber tubing to a flask containing phenol.

7. By means of a platinum loop, a beef infusion broth culture is planted with a loopful of material from each specimen. These broth cultures are incubated at 37° C. for 24 hours, and then examined for turbidity in the liquid.

8. These broth cultures indicate whether or not all extraneous organisms have been destroyed in the treatment process.

9. All the specimens showing no growth on the broth are then planted by a sterile capillary pipette on various types of media suitable for growing the tubercle bacillus.

10. After planting, the upper portion of the culture tube is flamed, the cotton plug is ignited, and inserted in the tube. A rubber cap (previously boiled for 15 minutes) is placed over the end of the tube.

* Report of the Referee and Associate for Tuberculosis and the Tubercle Bacillus for the Standard Methods Committee on Diagnostic Procedures and Reagents.

11. The specimen is incubated for 8 weeks at 37° C. The cultures are examined at the end of 4 weeks for visible growth. Cultures showing visible growth at this time are examined and reported. Cultures showing no growth after 4 weeks' incubation are reincubated for a further 4 weeks.

12. Many types of culture media have been used and the following have been found satisfactory: Lowenstein's, Petragnani's, Woolley's medium with and without glycerine, and modified Petroff's medium. Lowenstein's medium has been found to support growth the best.

Preparation Reagents—The reagents used for the treatment of these specimens, 3 per cent hydrochloric acid solution and the 3 per cent sodium hydroxide solution are sterilized by autoclaving 20 minutes at 15 lb. pressure; 0.08 gm. of the indicator bromcresol purple is added to the hydrochloric acid solution before sterilization. These reagents are then placed in a glass cabinet to exclude air-borne contaminants. A siphon apparatus with a bottle attached is placed in each flask. When not in use the glass case is kept closed.

MEDIA

Lowenstein's Culture Medium

Ann. de l'Inst. Pasteur, 50:161–166 (Feb. 13), 1933. See also *Tubercle,* IV:502–504 (Aug.), 1933.

1. 20 eggs carefully cleansed by immersion for ½ hour in tepid soapy water to which is added 5 per cent sodium carbonate

2. Asparagin solution: Asparagin (Merck) grammes 3; K_2HPO_4 (potassium phosphate, Merck) 1 gm.; Pure crystalline magnesium sulphate (Kahlbaum) 1 gm.; Pure glycerin 30 B. (Kahlbaum) 60 c.c.; Distilled water 1,000 c.c. This solution is sterilized for 2 hours in flowing steam. It is very stable.

3. Potato starch

4. Congo red (Grubler) 2 per cent aqueous solution sterilized in autoclave for 15 minutes

5. Malachite green (Grubler) 2 per cent aqueous solution sterilized in flowing steam for 2 hours

To 600 c.c. of the asparagin solution one adds 10 c.c. of a 50 per cent solution of potato starch and 60 c.c. of juice pressed from sterile tomatoes. This mixture is boiled for 2 hours in a water-bath. After cooling to 50° C., 16 eggs are added and then the yolk of 4 eggs. Then this mixture is actively stirred and 20 c.c. of Congo red solution is added.

After stirring, it is filtered through 4 layers of gauze. Then it is put in sterile tubes. Coagulation is produced by heating to 75–80° C. in flowing vapor. The tubes are carried to the oven and the next day it is again heated for 2 hours at 75–80° C. To cap the tubes use cellulose.

The culture media should be prepared oftener than every 2 weeks and kept in the dark.

Lowenstein's Media as Used in Studies Carried Out in the Ontario Department of Health:

Asparagin solution	1,000 c.c.
Potato flour	8.3 gm.
Tomato juice	100 c.c.

Boil in double boiler for 2 hours, cool to 45° C., and add:

Whole eggs	26
Egg yolks	8
2 per cent malachite green	34 c.c.

Strain through gauze, tube and sterilize.

Lowenstein's Medium with two dyes and sodium silicate is made as above, but with the addition of 34 c.c. 2 per cent Congo red solution and 0.5 c.c. of 1.9 per cent sodium meta silicate 9 H_2O per 100 c.c. of medium. This gives approximately 2 mg. SiO_2 per 100 c.c.

In making the asparagin solution, chemicals of any standard C.P. brand are used.

Tuberculosis Culture Media

Coagulated egg is the basis of many tuberculosis culture media. The coagulation and sterilization of these media cause considerable trouble. The following procedure is recommended:

The tubes of media in slant racks are placed in a cold autoclave. The racks are covered with newspapers to prevent too sudden contact with the steam. The autoclave is closed and the air outlet valve also closed. Pressure is raised to 15 lb. without letting the air escape. This condition is maintained for 10 minutes. The air outlet valve is then slightly opened (the pressure should not be allowed to vary more than ½ lb.), and the condensed water and some air are allowed to escape. All the air should not be allowed to escape. The valve is then closed and sterilization continued for 15 minutes.

Petroff's Medium

J. Exper. Med., 21:38–42 (Jan.), 1915.

Two parts of egg (white and yolk)
One part of meat juice
Gentian violet sufficient to the proportion of 1 to 10,000

Meat juice: 500 gm. of beef or veal are infused in 500 c.c. of a 15 per cent solution of glycerin in water. Twenty-four hours later the meat is squeezed in a sterile meat press and collected in a sterile beaker.

Eggs: Sterilize the shells of the eggs by immersion for 10 minutes in 70 per cent alcohol or by pouring hot water upon them. Break the eggs into a sterile beaker and, after mixing the eggs well, filter through sterile gauze. Add 1 part by volume of meat juice.

Gentian violet: Add sufficient 1 per cent alcoholic gentian violet to make a dilution of 1 to 10,000.

Tube about 3 c.c. in each sterile test tube and inspissate for 3 successive days: on the first day at 85° C., until all the medium is solidified, changing the places of the tubes if necessary; on the second and third days for not more than 1 hour at 75° C. For the bovine type omit the glycerin and infuse the meat for 24 hours in water. Bovine tubercle bacilli grow in this medium even if it contains glycerin, but on account of the popular belief and the lack of data we used a medium without the glycerin.

The following modification is used in the Laboratories of the Ontario Department of Health:

Milk	200 c.c.
Nutrient broth (not adjusted)	200 c.c.
Eggs	800 c.c.
Glycerin	60 c.c.
Malachite green 2 per cent (aqueous)	60 c.c.

NOTE: Nutrient broth—0.3 per cent beef extract, 0.5 per cent P.D. Peptone, 0.5 per cent sod. chloride

Mix well, stain through gauze, tube, slant and sterilize.

Herrold's Media as Modified by Feldman

J. Infect. Dis., 48:236, 1931.

Beef Extract Agar

Liebig's beef extract	3 gm.
Peptone	10 gm.
Sodium chloride	5 gm.
Agar	15 gm.
Distilled water	1,000 c.c.

Adjust media to pH 7.5.

Autoclave 15 minutes at 15 lb. pressure.

To each 150 c.c. of melted agar add the yolk of one egg, when temperature of agar is 60° C.

Allow agar egg yolk mixture to cool to 40° C., then dispense in tubes.

NOTE: Sterilize egg shell by immersion in phenol, or flame one end of the shell. By means of a pair of flamed forceps, make an opening. Allow the egg white to escape, then by means of a sterile pin or needle, puncture the yolk, allowing the yolk to mix with beef extract agar.

Potato Egg Medium for the Isolation of Tubercle Bacilli

J. S. Woolley and F. G. Petrik, *Am. Rev. Tuberc.*, Nov., 1931, pp. 596–603.

Autoclave medium sized unpeeled potatoes for 30 minutes at 15 lb. pressure. Peel and, after thorough mashing, suspend in 15 per cent glycerol-water, in the proportion of 500 gm. of original potato to 500 c.c. of 15 per cent glycerin. Heat in a boiling water-bath for 30 minutes, stirring occasionally. Strain through two layers of surgical gauze by compression into a flask and then boil filtrate for about 5 minutes. Cool and add 1 part of filtrate to 2 parts of well beaten whole fresh egg. Add sufficient 1 per cent crystal violet to make a concentration of 1 to 30,000. Stir thoroughly, to distribute the dye evenly throughout the mixture. Pour the mixture through one layer of sterile gauze into a tubing funnel and add approximately 7–8 c.c. of medium to each tube. Sterilize in the inspissator at the proper slant for ½ hr. at 85° C. on the first day, and at 75° C. on the second day.

Bring inspissator up to required temperature before introducing tubes. By omitting the crystal-violet an excellent medium is obtained for growing stock cultures of tubercle bacilli. Incubate the tubes to test for sterility. Tubes must be stored, seeded, and incubated in an upright position to keep the surface of the medium dry.

Modification used in the Laboratories of the Ontario Health Department:

Potato	1,000 c.c.
Water (no glycerin is used)	1,000 c.c.
Monobasic potass. phosphate	14 gm.

Filtrate from above.....	900 c.c.
Malachite green 2 per cent sol.	34 c.c.
Crystal violet 1 per cent sol.	6.5 c.c.

Petragnani's Medium

C. F. Cerruti, *Brit. J. Trop. Med. & Hyg.*, May, 1932, pp. 157–158.

Preparation of Medium

Milk	160 c.c.
Granular Potato starch (Fecula)	6 gm.
Peptone	1 gm.

Place these ingredients in a liter flask with pieces of a peeled potato the size of a hen's egg. Take the flask by the neck and put it in boiling water, shaking vigorously. When it has formed a colloid dough, stop shaking and leave in the boiling water-bath for 1 hour. Take it out and leave until the temperature is down to 60° C. Add 4 whole eggs and 1 yolk.

It is not necessary to sterilize the flask or milk, or to treat the eggshells. Now add:

Glycerin	12 c.c.
Malachite green 2 per cent aqueous solution	10 c.c.

Stir until it becomes a homogeneous mass and filter the mass through sterile gauze into a sterile flask; fill into sterile tubes (20 cm. by 22 mm.), or plates, using 12 c.c. to 15 c.c. of medium for each. Solidify in an inspissator in the following manner: Place the tubes or plates in the inspissator when it is still cold, bring it slowly to a temperature of 85° C., keep it at this temperature for 25 to 30 minutes, and leave to cool. This treatment is usually sufficient to solidify and to sterilize the medium, but its nutritive properties are not damaged by a tyndallization of 15 minutes at 80° C. the first day, and 10 minutes at 75° C. for two successive days.

The bovine strain will not grow well in the presence of glycerin, and when the material is suspected of containing a bovine strain two sets of cultures should be made, one with Petragnani's usual medium and one without glycerin, using bovine bile instead.

Modification used in the Laboratories of the Ontario Department of Health:

900 c.c. milk
36 gm. potato flour
6 gm. Peptone
6 pieces of potato (size of an egg)
24 eggs
6 egg yolks
72 c.c. glycerine
60 c.c. 2 per cent aqueous malachite green

Cut the potatoes into thin slices. Add milk, potato flour, and peptone. Cook in a double boiler for 2 hours, stirring until the mixture becomes sticky, then stir occasionally. Break the eggs and egg yolks into a 2 liter flask; shake well. Add the glycerine and malachite green and shake. Cool the potato milk mixture to 45°–50° C. Add egg glycerin mixture to the cooled potato mixture and mix well. Filter through gauze, tube and sterilize.

Egg Yolk for Growth of Tubercle Bacilli

Herrold. *J. Infect. Dis.*, 48:236.

Medium is as follows:

Beef extract agar prepared as follows:

Liebig's beef extract.....	3 gm.
Peptone	10 gm.
NaCl (mixture adjusted to pH 7.5)	5 gm.
Agar	10 gm.
Distilled water	1,000 c.c.

The yolk of 1 egg is added to 150 c.c. of melted agar at a temperature of 60° C. This is approximately 15 per cent of egg yolk. The agar is allowed to cool to about 40° C. and dispensed in tubes. A simple method of adding egg yolk is to flame one end of the egg and crack it with sterile forceps, so that a small opening is made in the shell for discarding the white, after which the yolk is broken with a sterile platinum loop to permit its flow into the container of melted agar. The egg may be immersed in phenol and then alcohol before flaming, or it may be placed in boiling water for 1 minute after preliminary flaming. The incubator should be kept moist to prevent drying of the plates, although it has been noticed that the yolk agar plates do not dry so quickly as blood agar plates. Closed containers may be used when the incubator is not sufficiently moist, or the plates may be wrapped with tin foil.

PREPARATION OF TUBERCULIN (KOCH'S A.T.)

Medium—

Asparagin (Pfanstiehl)	5.0 gm.
Ferric amm. citrate (Bakers)..	0.05 gm.
Mag. sulphate (Bakers).....	1.0 gm.
Pot. acid phosphate (Bakers)..	3.0 gm.
Amm. citrate (Bakers).....	5.0 gm.
Sodium chloride (Merck).....	2.0 gm.
Sodium carbonate (Merck)....	1.0 gm.
Glycerol (Bakers)	50.0 c.c.
Distilled water	1,000.0 c.c.

Dispensed in 150 c.c. amounts in 1 liter flasks. Sterilized in the autoclave at 120° C. for 1 hour.

Strain—Human strain of tubercle bacillus known as "Johnston." It is of moderate virulence such that 0.5 mg. of the moist culture will cause the death of the guinea pig in 6 to 8 weeks.

Time of Incubation—A small amount of the surface growth from a previous batch is transferred to each flask and allowed to remain at 37° C. for 4 months. At the end of this time the flasks are removed from the incubator, placed in an Arnold sterilizer and kept in free-flowing steam for 1 hour. The contents of the flasks are filtered through hardened filter paper (Whatman's No. 50). The filtrate is concentrated in a water-bath at 85° to 90° C. to one-tenth of the original volume of the medium. The concentrate is centrifuged at high speed, the supernatant removed and 0.3 per cent phenol is added and stored at 5° C.

TESTS FOR NONSPECIFIC TOXICITY, STERILITY AND STANDARDIZATION

Nonspecific Toxicity Test — Five c.c. are injected subcutaneously into a normal guinea pig and observed for 3 months, at the end of which time a complete autopsy is performed.

Sterility Test—Samples are taken from each container and tests made in fluid media according to the standards for sterility testing.

Standardization—A sample of the International Standard Old Tuberculin is received every 6 months from the Permanent Commission on Biological Standardization of the League of Nations, as distributed from Hampstead, London, and is used as the standard.

TEST FOR POTENCY

The Intradermal Test in guinea pigs is the method used for standardization. Six white guinea pigs of approximately 300 gm. are sensitized with human tubercle bacilli receiving 0.5 mg. of moist culture. Four weeks after the injection of the tubercle bacilli the hair is removed from the backs of the guinea pigs by using a fine electric clipper. The backs are then marked off into areas by means of an indelible pencil.

The following dilutions are made of (a) the Test Tuberculin and (b) the Standard Tuberculin: 1 in 500; 1 in 1,000; 1 in 2,000; 1 in 4,000. Each guinea pig is inoculated intradermally with 0.1 c.c. of each dilution of the two tuberculins; the dilutions of the Test Tuberculin along one flank and those of the Standard Tuberculin in corresponding sites of the opposite flanks. The reactions are read in approximately 24 hours after the injections and comparison made of the two tuberculins.

INTRADERMAL TESTS IN THE HUMAN OR THE MANTOUX TEST

The initial dose should be 0.01 mg. contained in 0.1 c.c. If negative the test should be repeated using 1.0 mg. in 0.1 c.c.

A. L. MACNABB, *Referee*
M. H. BROWN, *Associate Referee*

Tentative Methods for the Recognition of the Brucella Group of Organisms and Other Biological Procedures as They Relate to the Diagnosis of Undulant Fever*

I. Isolation Procedures
  A. Blood
    1. Citrated
    2. Inactivated
  B. Urine
  C. Feces
  D. Milk
  E. Guinea Pig Inoculation
II. Blood Serum Agglutination Tests
  A. Test tube method
    1. Preparation of antigens
    2. The test
  B. Rapid method
    1. Apparatus required
    2. Preparation of antigens
    3. The test
III. Milk Serum Agglutination Test
IV. Dye Bacteriostasis
  A. Description of media
  B. The tests
  C. Interpretation
V. Brucella Nucleoprotein Intradermal Test—Huddleson
  A. Preparation of Reagent
  B. Technic and Interpretation
VI. Opsono-Cytophagic Test
  A. The blood specimen
  B. Bacterial suspension
  C. The test
VII. References

Isolation Procedures

Blood Culture

Citrated Blood [1]—Receive blood in sterile rubber stoppered culture tube containing sufficient dry sterile sodium citrate to give a final concentration of 1 per cent per 10 c.c. A 10 c.c. sample is sufficient for culture. A 1 per cent concentration of sodium citrate will prevent phagocytosis of any organisms present in the blood.

Incubate the citrated blood sample in 10 per cent carbon dioxide at 37° C. Draw off a 0.3 c.c. sample every 3 days and run over the surface of a liver infusion agar slant. Reincubate the blood sample and examine all slants carefully for growth at the 3 day intervals. If the slants show the presence of contaminants, add all further samples of blood to liver infusion slants containing gentian violet in a 1:200,000 concentration. Make smears from suspicious slants and examine for Gram-negative cocco-bacilli. Continue this procedure until the specimen is used up or until a suspicious organism has been isolated. Isolate suspicious Gram-negative cocco-bacilli in pure culture on liver agar plates plus gentian violet and identify by agglutination, hydrogen sulphide production, and dye tests.

Maintain pure cultures of *B. melitensis* and *B. suis* on liver infusion slants in air and pure cultures of *B. abortus* on the same medium in 10 per cent carbon dioxide until they will grow in air.

Inactivated Blood [2]—The blood sample should be inactivated for 30 minutes at 55° C. immediately after it has been removed from the patient. Place the

* Report of the Referee on Serological, Bacteriological, and Other Biological Procedures in the Diagnosis of Undulant Fever, for the Standard Methods Committee on Diagnostic Procedures and Reagents.

sample in a flask containing 50 c.c. of liver infusion broth and incubate in 10 per cent carbon dioxide at 37° C. Observe at 3 day intervals, and run 0.3 c.c. sample over the surface of a liver infusion slant when the first sign of turbidity appears. Then follow the procedure as outlined under Citrated Blood. Incubate the blood sample at least 1 month.

URINE [1]

Centrifuge a 50 c.c. sample of urine at 3,000 r.p.m. for 1 hour. Spread the sediment over the surface of two liver infusion agar plates containing gentian violet in a 1:200,000 dilution. Incubate the plates in 10 per cent carbon dioxide at 37° C. for 72 hours and examine. Brucella colonies appear light blue violet in color on gentian violet plates. Transfer suspicious colonies to a second gentian violet plate if contaminants are present or to plain liver infusion agar if they are not. Identify the organism by agglutination, by hydrogen sulphide production, and by dye tests. Aerobic types of Brucella will not be inhibited in 10 per cent carbon dioxide.

FECES [3]

Mix 1 gm. of feces in 50 c.c. of sterile physiological salt solution and shake to obtain a thorough suspension. Filter the suspension through four layers of hospital gauze to remove gross particles. Centrifuge at 500 r.p.m. for 3 minutes to throw down other particles and other bacteria. Remove the supernatant suspension to another tube, and add sufficient agglutinating serum to make a dilution of 1:100. Shake and place the tube in a 37° C. water-bath for 2 hours. Centrifuge at 500 r.p.m. for 5 minutes and discard the supernatant. Resuspend the precipitate in physiological salt solution, shake and recentrifuge. Discard supernatant fluid. Repeat this procedure twice. Spread the precipitate over the surface of two eosin methylene-blue agar plates containing a 1:200,000 concentration of gentian violet. Incubate in 10 per cent carbon dioxide at 37° C. for 72 hours. Transfer suspicious colonies to a second gential violet plate if contaminants are present or to liver infusion agar plates if they are not. Identify the organism by agglutination, by hydrogen sulphide production and by dye tests. Aerobic types of Brucella will not be inhibited in 10 per cent carbon dioxide.

MILK

See *Standard Methods of Milk Analysis* for this procedure.

GUINEA PIG INOCULATION [1]

Guinea pigs are inoculated with, (1) blood, (2) urine sediment, and (3) milk.

Blood—Inoculate one guinea pig subcutaneously with 2 c.c. of blood from specimen received for culture. At the end of 8 weeks, anesthetize the animal and withdraw sufficient blood for an agglutination test. Kill the animal and culture the spleen pulp, heart's blood, urine, and bile on liver infusion agar. Make duplicate plates for each specimen. Incubate one set in 10 per cent carbon dioxide and the other in air at 37° C. Observe the plates at 3 day intervals for suspicious colonies. Make smears and examine for Gram-negative cocco-bacilli. Identify as indicated under Identification Procedure.

Urine — Centrifuge uncatheterized specimens of urine and follow the procedure for Blood. Inject animals with 2 c.c. specimens of catheterized urine.

Milk—See *Standard Methods of Milk Analysis* for this procedure.

BLOOD SERUM AGGLUTINATION TEST *With Unknown Serum*

TEST TUBE METHOD [1]

Preparation of Antigen—Prepare a monovalent antigen with a type strain of *B. abortus*. Inoculate liver infusion agar Blake bottles with saline suspensions of 72 hour liver infusion agar cul-

tures of the organism. Incubate the Blake bottles in air at 37° C. for 72 hours. Remove the growth from the agar surface with a solution of 0.5 per cent phenol in 0.85 per cent salt solution. Make smears to determine purity of suspensions. Filter suspensions through several layers of hospital gauze to remove particles of agar. Unheated antigens are satisfactory and are recommended. Store the concentrated antigen until ready for use. For routine tests, dilute the antigen concentrate with phenolized salt solution to a turbidity 7 cm. Gates. Diluted antigen will remain satisfactory at room or coldroom temperature for 1 year. Check the antigen with known antisera of high, medium and negative titer.

The test—Bring samples and antigen to room temperature. Prepare serum dilutions on the multiple or decimal system. Add antigen and shake tubes to mix serum and antigen. Incubate in a water-bath at 55° C. for 4 hours and hold at room temperature or in the coldroom over night before reading. Record complete agglutination by a plus sign (+), incomplete agglutination with a plus-minus sign (±) and negative agglutination with a negative sign (—).

RAPID METHOD [1]

Antigen dropper pipette—Prepare capillary pipettes by drawing out glass tubing ⅛" inside diameter. Cut the capillary end at a diameter 0.07". Do not flame delivery end of pipette. This pipette should deliver 0.03 c.c. antigen in each drop.

Glass plate and darkfield illumination box—Rule of a 6½" x 14" piece of double thickness window glass in 1" squares with a diamond point pencil. Set the glass on the top of a box 12" long x 10" wide x 6" deep with one side of the top covered to a width of 3½". Place a light bulb under the covered portion of the top to provide direct illumination. Paint the inside of the box directly behind and above the light white and the remainder black.

Serum amounts—Use 0.08, 0.04, 0.02, 0.01 and 0.004 c.c. of serum in the test. These volumes, when added to 2 c.c. of antigen in the tube test give final dilutions of 1–25, 1–50, 1–100, 1–200 and 1–500.

Preparation of Antigen—See "Brucella Infections in Animals and Man," by I. Forest Huddleson, for detailed procedures for the preparation and standardization of rapid antigen.

The test—Bring samples of serum and antigen to room temperature. Shake antigen thoroughly and at 2 hour intervals during sample testing. Test 6 sera at one time; 0.08, 0.04, 0.02, 0.01 and 0.004 c.c. amounts of each serum to be tested in each row marking from the bottom to the top of the row. Place a drop of antigen on each drop of serum. Hold the pipette vertical so that the volume of the drop delivered will be correct. Mix the serum and antigen for each serum with a fresh toothpick working from the bottom to the top of each row. Lift the plate and tilt back and forth for 2–3 minutes. Place plate back on box, turn on light, and read results. Results may be read in serum amounts or serum dilutions. Turn off light as soon as results are read. Scrub the plate with washing powder, rinse in tap and distilled water and wipe dry with a clean chamois skin.

With Unknown Organism

TEST TUBE METHOD

Use the same procedure for agglutination as outlined under Agglutination Test, with unknown serum. Use an abortus antiserum [4] prepared as follows: Prepare antisera in rabbits. Dilute a suspension of an avirulent strain of *B. abortus* to a turbidity of 7 cm. with the Gates apparatus. Kill the suspension by heating for 1 hour at 56° C. Inject 1 c.c. of suspension subcutaneously on 2 consecutive days. On the third date in-

ject 0.5 c.c. intravenously. One week from the first injection inject 1 c.c. of heated suspension intravenously. Repeat this dose for the next 2 days. One week from the fourth injection inject 1 c.c. of heated suspension intravenously. Inject the live saline suspension from one-half of a liver infusion agar slant intravenously daily for the next 2 days. Rest the animal 1 week, and inject the live saline suspension from one-half of a liver infusion agar slant intravenously. Inject the live suspension from one whole slant intravenously daily for the next 2 days. Rest the animal for one week. Inject the live suspension from one whole slant intravenously daily for 3 days. Make test bleedings to determine the titer and bleed the animal to death from the heart if the titer is satisfactory. If not, repeat the schedule of the last week until test bleedings indicate a satisfactory titer. The schedule produces an antiserum with a titer of 1 to 2,560.

MILK SERUM AGGLUTINATION TEST [5]

Let the samples of milk stand for 6 to 8 hours to allow cream to separate. Pipette off as much cream as possible and place samples in tubes. Add 2 drops of rennet to each 5 c.c. portion of milk and mix thoroughly. Incubate tubes at 37° C. in a slanting position so that the curd and serum will separate. The serum will form in about 2 hours. The samples should be placed in a cold-room for 6 to 8 hours to obtain sera free from particles of casein. Test the clear serum in the same manner as blood serum. Do not test sour milk, colostrum, or milk in which the curd has become partially digested. Samples of milk should be iced if they are to be in transit more than 10 hours.

DYE BACTERIOSTASIS [1]

Liver Infusion Agar—See preparation of medium under Determination of Hydrogen Sulphide production.

One per cent stock thionin solution, aqueous—

Use the following formula to prepare a 1.0 per cent stock solution:

a. Formula

$$\text{Certified thionin } 1 \times \frac{1}{\text{total dye content}}$$

Distilled water up to 10.0 c.c.

b. Procedure

Weigh dye and add to distilled water in a culture tube. Boil for 15 minutes in a water-bath.

One per cent basic fuchsin solution—

Use the following formula to prepare a 1.0 per cent stock solution:

a. Formula

$$\text{Certified basic fuchsin } 1 \times \frac{1}{\text{total dye content}}$$

Distilled water up to 10.0 c.c.

b. Procedure

Weigh dye and add to distilled water in a culture tube. Boil for 15 minutes in a water-bath.

Thionin liver infusion agar plates—Melt French squares of liver infusion agar in flowing steam or water-bath. Heat stock solution of thionin for 20 minutes in flowing steam or water-bath. Add sufficient 1.0 per cent thionin solution, *while still hot*, to each bottle of liver infusion agar to make a dye concentration of 1 to 50,000. Whirl bottles to mix thoroughly and pour into sterile Petri dishes.

Fuchsin liver infusion agar plates—Melt French squares of liver infusion agar in flowing steam or water-bath. Heat stock solution of basic fuchsin for 20 minutes in flowing steam or water-bath. Add sufficient 1.0 per cent basic fuchsin solution, *while still hot*, to each bottle of liver infusion agar to make a dye concentration of 1 to 25,000. Whirl bottles to mix thoroughly and pour into sterile Petri dishes.

The test—Divide each thionin plate into equal segments. Divide each fuchsin plate into equal segments. Spread a heavy loopful of each culture over one

segment of one thionin and one fuchsin plate. Incubate plates for 72 hours at 37° C. and record presence or absence of growth as indicated below under Recording. Care should be taken not to confuse a heavy inoculum for growth.

Recording—Indicate results according to the following key:

Growth	*Symbol*
Present	+
Absent	—

Classification—Classify the strain according to the following key:

Growth		
Thionin	*Basic Fuchsin*	*Classification*
+	+	*B. melitensis*
—	+	*B. abortus*
+	—	*B. suis*

HYDROGEN SULPHIDE PRODUCTION [1]

Lead acetate paper—Prepare strips of lead acetate paper as follows: Dissolve 10 gm. of lead acetate normal, C.P. grade, in 50 c.c. of boiling distilled water. Immerse sheets of filter paper in the acetate solution until saturated. Dry paper in incubator at 37° C. Cut paper in strips measuring 2½" x ¼". Store in stoppered bottles.

Liver infusion agar—Prepare liver infusion agar slants according to the following formula:

a. Formula

Agar, Bacto	20 gm.
Tap water	500 c.c.
Liver infusion	500 c.c.
Peptone, Bacto	10 gm.
Sodium chloride, C.P.	5 gm.

b. Procedure

Grind fresh, fat-free beef liver to a plastic mass. Mix 1 lb. of ground liver with 500 c.c. of tap water. Cover the container and place in flowing steam for 20 minutes. Remove cover and stir to mix thoroughly. Continue heating for 1½ hours. Filter through 30 mesh wire screen. Add ingredients in above formula to 500 c.c. of liver infusion. Steam for 1 hour. Cool to 60° C. and adjust to pH 7.0. Steam for ½ hour. Decant and tube in 5 x ¾" tubes. Autoclave tubes for 25 minutes at 121° C. The pH should drop to 6.6 during sterilization.

c. Checks

Incubate for 48 hours at 37° C. to check sterility.

The test—Inoculate a liver infusion agar slant with a large loopful of each culture to be tested. Insert a strip of lead acetate paper between the cotton plug and tube wall so that the strip projects 1" below the bottom of the cotton plug. Incubate at 37° C. for 24 hours. Record the absence or presence of blackening on the lead acetate paper as indicated below under Recording. Remove the strip of lead acetate paper and insert a fresh strip. Save each lead acetate paper so that final interpretation may be made from the four papers for each strain. Incubate at 37° C. for 24 hours and record results. Repeat this procedure until the production of hydrogen sulphide has been observed for 4 successive days.

Recording—Record results under "H_2S Production" of report form. Record the H_2S production for the first 24 hours under 1, for the second 24 hours under 2, and so on. Record results according to the following key:

H_2S Production	*Symbol*
None	—
Trace	Tr.
Moderate to marked	+

Classification—

Classify each strain according to the following key:

H_2S Production	*Classification*
None or trace for 4 days	*B. melitensis*
Moderate to marked for the first 2 days	*B. abortus*
Moderate to marked for 4 days	*B. suis*

BRUCELLA NUCLEOPROTEIN INTRADERMAL TEST—HUDDLESON [1]

Preparation—Grow a smooth strain of *Br. abortus* separately for 72 hours at

37° C. on liver infusion agar in monel metal trays 6" wide, 10" long and ½" deep. The tops of the trays are fitted with a cover of the same metal, but slightly larger in size. At the end of the incubation period lift the growth from the surface of the medium with a celluloid spatula and suspend in a large volume of distilled water. Recover the cells immediately in a Sharples laboratory centrifuge and dry in vacuum over H_2SO_4 at 37° C. The procedure used in obtaining the nucleoprotein fraction from the dried cells was chosen for the purpose of preventing as far as possible chemical alteration of the protein. Carry the separation out rapidly at 5° C. and with suitable precautions to prevent enzymatic decomposition.

Grind a 100 gm. portion of the dried cells in a ball mill to a fine white powder and extract at room temperature for two 5 day periods each with 500 c.c. of anhydrous ether. Decant the solvent and collect the residue on a Buchner funnel and wash with ether. Transfer the air-dried residue from the ether extraction to a small porcelain ball mill together with 200 gm. of cracked ice made from distilled water, and rotate the mill for 2 hours. During the period of rotation add another 100 gm. of ice to maintain a low temperature. At the end of the rotation period add 300 gm. of cold water and allow the mill to stand for 5 hours in the icebox. Shake occasionally by hand. Decant the contents of the mill, the stiff foam being cut with ether. Grind the remaining residue in the mill for another hour with 200 gm. of cracked ice and water and wash from the mill with cold water.

Make the total combined volume of the extracts from the mill to 2 liters. Pass the combined extracts through a Sharples centrifuge several times to separate the cellular material. The final liquid should be clear and slightly yellow. Precipitate the clear extract at a pH of 3 by the addition of 1:1 acetic acid. Allow precipitation to continue for 24 hours in the cold. Separate the precipitate from the supernatant liquid by decantation and centrifuging. Suspend in cold water by drawing it through a fine cloth by suction. Stir the suspended material mechanically and cool with ice while N1 NaOH is added from a burette to alkalinize and effect solution. Keep the pH below 11. Precipitate the solution with 1:1 acetic acid and redissolve in slightly alkaline water three times. Reprecipitate the alkaline solution with 1:1 acetic acid; wash the precipitate twice with distilled water and then dehydrate first with alcohol, then with acetone and ether. Dry the grayish white residue in vacuum over sulphuric acid. Solution of the dried residue is effected in physiological salt solution brought to a pH of 7.4 with N1 NaOH. Sterilize the solution by passing through a sterile Berkefeld W candle. The recovered filtrate is clear and colorless. It is now ready for standardization based on total nitrogen content. The sterile recovered liquid should be diluted with sterile physiological salt solution so that 0.1 c.c. (one skin test dose) contains approximately 0.000015 gm. of nitrogen. It may be placed in sterile vials and sealed until ready for use. The diluted nucleoprotein fraction appears to retain its potency for an indefinite period.

Technic and Interpretation—Make the test by injecting 0.1 c.c. of the fluid intracutaneously in the lateral surface of the forearm, using a 26 gauge needle. The size of the local reaction, which is characterized by a circumscribed erythema and slight edema, may vary from 1 to 3" in diameter. Read the reaction 24 hours after injection. It may persist for 48 to 96 hours. There is rarely, if ever, any necrosis or sloughing of the tissue at the point of the local reaction. In the infected, the local reaction may be accompanied by a more marked manifestation of the present

symptoms. Those that are hypersensitive will show a severe systemic reaction along with the local reaction. Those that have not been sensitized to Brucella and who are probably susceptible to infection show no local or systemic reaction. Often one sees in certain normal individuals an erythema about ½" to 2" in diameter with no edema around the point of the injection. It has the appearance of a nonspecific reaction.

OPSONO-CYTOPHAGIC TEST [1]

Blood Specimen—Collect a 5 c.c. sample of blood in a sterile vial containing sufficient sodium citrate to make a concentration of 0.8 per cent. Keep the specimen in the coldroom until the test is made. Blood samples must be examined within 6 hours to prevent the disintegration of the polymorphonuclear leucocytes.

Bacterial Suspension—Prepare a fresh suspension daily from a 48 hour liver infusion agar culture. Suspend several loopfuls in saline at pH 7.0 to a turbidity of 2 mm. Gates. Suspensions of any of the three species of Brucella are satisfactory so long as they do not become fast to ingestion. Test cultures to "fastness" against immune citrated blood.

The test—Place 0.1 c.c. of whole blood and 0.1 c.c. of bacterial suspension in a clean culture tube. Mix the contents thoroughly and incubate at 37° C. for 30 minutes. Do not resuspend the cells after incubation. Place a small drop of cells on the end of a clean glass slide with a fine capillary pipette. Spread the drop with a second slide as in the preparation of a blood smear. Dry the slide rapidly before an electric fan. Cover the slide with Hastings solution for 15 seconds, then add 1 c.c. of distilled water. At the end of 10 minutes wash the slide free from stain with distilled water. Dry the slide before the electric fan.

Degree of Opsono-cytophagic activity—Count 25 polymorphonuclear leucocytes and record the degree of phagocytosis as follows:

Negative . . . . . no phagocytosis
Slight . . . . . 1 to 20 bacteria per cell
Moderate . . 21 to 40 bacteria per cell
Marked . . 40 or more bacteria per cell

Compare the degree of phagocytosis with the results of the intradermal test and the agglutination test in order to arrive at a diagnosis.

The following system of diagnosis has been proposed by Huddleson:

A PROPOSED SYSTEM FOR THE DIAGNOSIS OF UNDULANT FEVER ACCORDING TO THE RESULTS OF THE AGGLUTINATION, ALLERGIC, AND OPSONO-CYTOPHAGIC TEST

Agglutination Test	*Allergic Skin Test*	*Opsono-Cytophagic Power of Blood*	*Status Toward Brucella*
—	—	Zero to 20 per cent of cells, slight	Susceptible
—	+	Zero to 40 per cent of cells, marked	Infected
+	+	Zero to 40 per cent of cells, marked	Infected
—	+	60 to 100 per cent of cells, marked	Immune
+	+	60 to 100 per cent of cells, marked	Immune

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G. D. CUMMINGS, *Referee*

Tentative Methods for the Bacteriological Diagnosis and Control of Whooping Cough*

I. The Cough Plate Technic for Isolating *B. Pertussis*
- A. Medium
- B. Exposure of cough plates
- C. Incubation and examination of plates
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II. Biochemical Characteristics of an Animal Reaction to *B. Pertussis*
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The Cough Plate Technic for Isolating B. Pertussis

Cough Plate Medium

The cough plate[1] used for the isolation of *B. pertussis* is prepared with a modification of Bordet and Gengou's potato blood agar.[2]

Base:

Peeled sliced potatoes....	500 mg.
Glycerine, U.S.P.	40 c.c.
Distilled water	1,000 c.c.

Boil the potatoes in glycerine and water until soft. Make up to volume, strain through gauze and allow to stand for sedimentation. Siphon off the supernatant liquid.

To 500 c.c. clear potato extract add:

Sodium chloride solution, 0.75%	1,500 c.c.
Agar, Bacto	60 gm.

Let stand for 15 minutes to saturate the agar. Heat until the agar is dissolved and dispense in amounts convenient for storage. Autoclave for 25 minutes at 15 lb. pressure (120° C.). This base may be stored indefinitely.

Finished medium—To the melted base, at 45° C., add blood to make a final concentration of at least 15 per cent. The blood should be used when fresh, never more than 72 hours after it has been obtained. It may be from sheep, horse, or human source, but avoidance of horse blood for the vaccine medium is recommended. Mix the blood with the base by whirling, and pour into plates—about 15 c.c. per Petri plate. Either glass plates or metal boxes may be used.

Checks — Incubate several plates from each lot made for 24 hours at 37° C. to check sterility. Inoculate one plate with *B. pertussis* to check the growth promoting ability, colony appearance, and characteristic hemolytic zone.

Satisfactory plates should be bright cherry red in color, and free from bubbles and lumps of agar. They may

* Report of the Referee on Methods and Reagents for the Diagnosis and Control of Whooping Cough for the Standard Methods Committee on Diagnostic Procedures and Reagents.

be used as long as they remain moist and red, usually 2 to 3 weeks if kept in the cold room.

EXPOSURE OF COUGH PLATES

Hold the uncovered plate 4 or 5 inches from the patient's mouth during several expulsive coughs. If necessary, induce a cough by tickling the throat with a swab, pressing on the larynx, giving a drink of ice water, or some other device. Cover the plate as soon as possible after obtaining cough in order to avoid contaminants from the air.

INCUBATION AND EXAMINATION OF PLATES

As soon as possible after the plates have been inoculated, place them in the incubator at 37° C. Examine them several times during the first 48 hours for molds or other spreading colonies which might overgrow the plate, and cut these out with a sterile needle or scalpel. After about 48 hours, search for colonies of *B. pertussis* using a hand lens. Examine the glass plates by transmitted light, placing them uncovered over an ordinary substage lamp. Examine the aluminum plates by reflected light, placing the lamp on end and tilting the uncovered plate in front of the light which will come from the side. If such plates are questionable, transfer the medium to a glass Petri plate and examine by transmitted light. Examine all plates twice daily until *B. pertussis* is found or the negative ones until they have been discarded—6 days after they were received.

CRITERIA FOR IDENTIFICATION OF B. PERTUSSIS COLONIES

Colony appearance—*B. pertussis* colonies appear smooth, raised, glistening, pearly and almost transparent, while the colonies of Gram-positive cocci in general appear duller, darkly colored, and opaque. They are surrounded by the characteristic zone of hemolysis—a zone which is not sharply delimited but merges somewhat diffusely into the surrounding medium.

Consistency of the growth—The consistency of the growth and the manner of its diffusion into water are typical of *B. pertussis*. As a loop of growth touches a drop of water or salt solution, it spreads and shows at first a momentary clumping effect but with very slight agitation it quickly smooths into a homogeneous suspension.

Morphology and staining reaction—Stained by Gram's method, *B. pertussis* decolorizes readily—much more readily, in fact, than does *H. influenzae*. Viewed microscopically, the small, faintly-stained coccoid bacilli are scattered evenly throughout the film, occurring singly for the most part, seldom in chains of even 2, and not found as pleomorphic threads. Frequently they show bipolar staining.

Agglutination tests — Questionable colonies may be tested by a *slide agglutination* technic. Suspend the colonies to be tested in a drop of saline on a slide and mix a loopful of this suspension with a loopful of *B. pertussis* antiserum diluted 1:10. Observe for immediate agglutination, in comparison with the remaining suspension to which serum was not added. For larger amounts of culture and for quantitative results use the *rapid agglutination technic*, described later.

Identification by sub-culture—If a questionable colony cannot be identified with certainty, make a sub-culture to a cough plate, spreading the colony over a small area. There will usually be sufficient growth after 24 hours' incubation for a slide agglutination test and stained preparation.

REPORTING THE FINDINGS

Positive plates may be reported as soon as found. Plates showing no colonies of *B. pertussis* on the 4th day

may be reported as negative to date. The plates should be examined for 2 more days, and additional reports made if any plates are found positive.

BIOLOGICAL CHARACTERISTICS AND REACTIONS IN ANIMALS

To encourage antigenic stability of the cultures (see references 3, 4, and 5), they should be kept on Bordet-Gengou medium containing at least 15 per cent blood. When adapted to other media such as the ordinary blood or coagulated blood agar, brain-veal, or plain infusion agar, the cultures are likely to change antigenically and also they are more difficult to maintain. In this outline we are concerned more particularly with Phase I cultures—the term used by Leslie and Gardner to describe the antigenic state characteristic of cultures just isolated from patients with whooping cough.

The *colony appearance* on Bordet-Gengou medium and the *staining characteristics* of *B. pertussis* have been described above under criteria for identification of *B. pertussis* colonies. It may be added that capsules can be demonstrated by proper technic. For this, Lawson [4] developed a method in which Wright's stain is used. The method of Churchman and Emelianoff [9] also gives good results.

Cultivation and biochemical reactions [4, 5, 6, 8, 14]—*B. pertussis* is a strict aerobe and grows best at a temperature of 37° C. Some formula based upon Bordet-Gengou potato-glycerine blood agar seems best suited of all known media to meet the growth requirements.

When adapted to certain other media, the growth is characteristic. On *coagulated blood agar*, after several preliminary transfers with heavy inoculum the growth is fairly profuse but after a few days tends to adhere to the medium and to die out in a relatively short time. In shallow layers of *broth medium*, a viscid, ropy sediment with floating strands, is produced. On *potato slants*, the growth becomes tan colored after several days' incubation, in this respect resembling *B. bronchisepticus* although the color change is less marked. *Litmus milk* is made alkaline after a week or more. On *carbohydrate media* there is no reaction. *Indol* is not formed. *Nitrates* are not reduced.

Serological reactions—Phase I cultures form a homogeneous group with respect to agglutination and complement fixation reaction.[2, 6]

The *rapid agglutination technic* [10] is particularly adapted to the study of *B. pertussis* and is given here:

Antigen preparation—With a stiff bent needle, remove the 48 hour growth of *B. pertussis* on Bordet-Gengou medium and suspend in saline. Filter the suspension through a thin layer of cotton to assure a smooth, satisfactory antigen. Adjust the turbidity to approximately 20 billion organisms per c.c., after comparing a diluted sample with a series of known suspensions—a convenient series includes 1, 2.5, 5, and 10 billion organisms per c.c., respectively.

Note: Have ready a supply of test tube cotton filters, prepared as follows: Shape a thin layer of moistened absorbent cotton around the finger, place on the bent end of a strip of copper wire netting as a support, and insert in a test tube of convenient size—with the free end of the wire strip hooked over the lip of the tube. Shape the cotton against the walls of the tube with a wooden applicator, plug the tube and sterilize. After use for filtration, remove and discard the filter—re-inserting the sterile plug.

For larger filters for use in flasks, wrap the cotton around the outside of a square of wire netting cut from a bias strip and shaped to fit inside the neck of the flask. Cover this with a single thickness of gauze. Suspend the filter by two wires which cross under it, pass through the netting, and hook over the lip of the flask.

Preparation of antiserum—Immunize a rabbit against a Phase I culture of *B. pertussis*, using a 10 billion suspension of a 48 to 72 hour culture on Bordet-Gengou medium, killed by contact with merthiolate 1:10,000, 48 hours or more in the cold room. Inject 3 doses intravenously—0.4, 0.8, and 1.0 c.c., respectively, per kg. weight, at 3 or 4 day

intervals. Make a trial bleeding about 1 week after the third injection. If the agglutination titer (" equivalent " as described below) is approximately 1:20,000 with the homologous culture and with several other recently isolated strains, bleed the rabbit from the heart or carotid. Inactivate at 56° C. for 30 minutes.

Dilution of the antiserum—A scheme for diluting the serum such as follows is convenient for a series of tests in which no more than 2 c.c. of each serum dilution will be required.

Dilutions lower or higher, or in a closer range, and smaller or larger quantities may be prepared as indicated.

The agglutination test—Mix 0.1 c.c. of each serum dilution with 0.1 c.c. of antigen, the measurements being made with 1 c.c. pipettes graduated in 10ths. For an antigen control, mix 0.1 c.c. of saline with 0.1 c.c. of antigen.

A CONVENIENT DILUTION SCHEME

Tube No.	*Serum Dil. Required*	*To Make Serum Dilution*		
		Serum Dil. to Be Used	*Serum c.c.*	*Saline c.c.*
1	1:10	undiluted	0.3	2.7
2	1:50	1:10	1.0	4.0
3	1:100	1:25	2.5	2.5
4	1:250	1.100	1.0	1.5
5	1:500	1:100	0.5	2.0
6	1:750	1:100	0.5	3.25
7	1:1,000	1:100	0.7	6.3
8	1:1,500	1:1,000	2.0	1.0
9	1:2,000	1:1,000	1.0	1.0
10	1:2,500	1:1,000	1.0	1.5

Shake the mixture by hand for 3 minutes, rocking the racks at the rate of approximately 60 back-and-forth motions per minute, and in such a way that the contents flow about three-quarters of the length up the tube.

After the period of shaking, add physiological salt solution to facilitate reading. The Hipple pipetting apparatus, used in the Kahn test, and set to deliver 0.5 c.c. of saline is convenient for large series of tests.

Reading the results—Read the reactions immediately after adding saline. Record each tube as —, ±, +, ++, +++ or ++++, according to the degree of agglutination. In determination of a titer, ++ is the lowest reading to be considered as an end point.

Interpretation of results—Since in the literature agglutination titers are apt to be expressed in terms of the usual 1 c.c. test, it is important to reduce the quantities of reagents used in the two tests to equivalent terms.

In the 1 c.c. test a culture suspension of about 4 billion per c.c. is required and 0.5 c.c. of this is mixed with 0.5 c.c. of diluted serum. In the 0.2 c.c. rapid test, 0.1 c.c. of a 20 billion per c.c. suspension is mixed with 0.1 c.c. of serum. That is, in testing any particular serum dilution, one-fifth as much serum is used in the rapid test as is employed in the 1 c.c. test with the same dose of antigen. This means that on the basis of the actual amount of serum present in the dilution being tested, any particular final dilution in the rapid test is equivalent to 5 times that final dilution in the other test. For example: a series of serum dilutions 1:10 to 1:2,500 in the rapid test gives a series of final dilutions 1:20 to 1:5,000, which corresponds to a series of final dilutions in the 1 c.c. test of 1:100 to 1:25,000.

Reactions in animals—For reports of transmission of pertussis to monkeys by the injection of *B. pertussis* cultures see references 15, 16, and 17. In certain other animals, characteristic reactions follow injection of the culture and constitute helpful criteria in the study of *B. pertussis*.

A *skin reaction* is produced in rabbits and guinea pigs by the intracutaneous injection of a living suspension of *B. pertussis* Phase I. Within a few hours after injection there is an ischemic indurated area at the site of injection. By 24 hours a purplish center appears, surrounded by an

ischemic ring and beyond that, the outer part of the indurated zone appears inflamed and may show an arborization of capillaries. The central zone develops into a definite dark purple necrotic area by the end of 48 hours. After a few days, depending upon the intensity of the reaction, a scab takes the place of the purple zone, and later a scar indicates the site of inoculation. A dose of 0.1 c.c. of a 1 billion per c.c. suspension gives a reaction about 1 cm. in diameter.

Intraperitoneal injection of mice and guinea pigs with a suitable dose of *B. pertussis* Phase I is followed by death and characteristic gross pathology at autopsy. The killing dose shows considerable variation with individual animals. Usually a dose of 1 c.c. of a 5 billion per c.c. suspension kills a mouse within 72 hours. Twice this dose is usually required for guinea pigs. At autopsy there is observed a more or less extensive hemorrhagic area in the peritoneum at the site of injection, and occasionally an area of necrosis in the skin. The peritoneal fluid is increased—markedly so in the guinea pig. Frequently all organs and membranes are covered with a sticky mucoid exudate. Grossly, heart, lungs, liver and spleen show no changes. There is marked congestion around the reproductive organs. *B. pertussis* may be recovered with ease from the peritoneal fluid. Cultures from the heart's blood of the mice usually show *B. pertussis*.

B. Pertussis Vaccine

Choice of Cultures

For each lot of vaccine, select several Phase I pertussis cultures which have been kept only on Bordet-Gengou medium containing at least 15 per cent blood. Just prior to their use, the cultures should be checked for Phase I characteristics[11]: typical colony appearance on Bordet-Gengou medium, including hemolysis of red cells; typical morphology and staining reactions; agglutination with Phase I antiserum; pathogenicity for mice; and skin reaction of hemorrhagic necrosis in the rabbit.

Preparation of Vaccine[11, 12, 13]

B. pertussis vaccine medium—Use the cough plate medium previously described.

Inoculation of the medium—Prepare seed flasks by inoculating 500 c.c. French square bottles, each with 3 c.c. of a 10 billion per c.c. suspension in broth or salt solution of a 48 to 72 hour growth of *B. pertussis*. If larger flasks or bottles are used, vary the inoculum accordingly. Tilt the bottles until the entire surface is covered with the inoculum. After the seed flasks have incubated for 48 to 72 hours, suspend the growth in broth or salt solution and check for purity by Gram-stained preparation. Using the seed suspension, inoculate 1,000 or 500 c.c. bottles, 3 c.c. per 500 c.c. bottle. Twenty-five to 30 bottles make a reasonable number for one planting and one 500 c.c. seed bottle will afford sufficient inoculum for 6 French squares of 500 c.c. capacity.

Incubation—Incubate at 37° C. for 72 hours.

Harvesting—Discard any obviously contaminated bottles before beginning harvest. Wash off the growth on the surface of the medium with physiological salt solution using 3 or 4 c.c. per bottle. To loosen the growth, use sterile glass rods bent to reach the corners of the bottles. Make Gram-stained preparations from each bottle and examine for purity of the growth. Discard or study further by sub-culture any bottles suspected of contamination. Pool the suspensions from the remaining bottles. Wash off the residuum of growth with an additional 2 or 3 c.c. of salt solution. For 1 liter of vaccine,

about 50 bottles of 500 c.c. capacity are required.

Washing the suspension and adding the killing agent—By the addition of salt solution, adjust the pooled suspension to compare roughly with the turbidity of a known 10 billion suspension. Centrifugate in 250 c.c. centrifuge bottles for 1 hour, or until the supernatant is fairly clear. Discard the supernatant and resuspend the sediment in saline containing merthiolate 1:10,000 or phenol 0.5 per cent. The volume of this suspension should be somewhat less than that required for a 10 billion suspension to allow for dilution during standardization.

Filtering—Using a cotton and gauze filter (described under the agglutination technic), fitted into the mouth of a flask, filter the suspension to remove particles of medium and insure a smooth, even product. Wash the filter with about 10 c.c. saline to reduce loss.

Killing the organisms—Allow the suspension which contains merthiolate 1:10,000 or 0.5 per cent phenol to remain at cold room temperature for at least 1 week. Shake several times daily.

Sterility and purity tests—After the period in the cold room spread 0.1 c.c. on a Bordet-Gengou plate streaking out on half the plate. Inoculate fermentation tubes containing 25 c.c. of dextrose broth with 0.5 c.c. and 0.2 c.c. respectively. Incubate at 37° C. for 5 days.

Make Gram-stained preparations and examine for purity of the suspensions.

Standardization of turbidity—Remove 5 c.c. of the suspension from the flask and compare the turbidity with that of a known 10 billion suspension. See "Turbidity Standards" below. If the suspension to be standardized is markedly denser than the standard, dilute the entire lot to approximately 10 billion by the addition of salt solution plus merthiolate 1:10,000. Make 1:2, 1:4, and 1:5 dilutions of the adjusted suspension in vials of the same diameter as the standards and compare with the 5, 2.5, and 2 billion standards respectively. Slight differences in turbidity can be more easily detected if a printed sheet is held behind the vials, and the suspensions examined against a bright light. Dilute the lot as indicated by these comparisons, and re-check the final suspension with a second series of dilutions.

Turbidity standards—Prepare a suspension of *B. pertussis* as for a vaccine. Adjust to 10 billion per c.c. by a series of direct counts in a Petroff Hauser chamber. Check this suspension by a Hopkins tube determination as follows:

Place 5 c.c. of the suspension in a Hopkins tube and centrifugate at 2,800 r.p.m. for 30 minutes. At this time the supernatant should be clear and the organisms packed in the narrow graduated portion of the tube. If this has not occurred, centrifugate for an additional 10 minutes. Determine the amount of packed organisms in the tube as indicated by the graduations. If the two methods check, the sediment will represent 0.3 per cent of the volume. Occasionally a suspension is encountered which does not pack well in a Hopkins tube. Such a suspension should not be chosen for use as a standard.

Make 1:2, 1:4, and 1:5 dilutions of the 10 billion suspension, corresponding to 5, 2.5, and 2 billion per c.c. respectively. Seal 5 c.c. amounts of these standard suspensions—four including the 10 billion—in vials. They will keep for an indefinite period.

Filling the vials—Dispense the vaccine as desired. For individual injections, 1.5 c.c. quantities in 2 c.c. ampules are convenient; for clinic use, larger quantities are more satisfactory.

TEST OF THE FINAL PRODUCT

Sterility and purity—Test sterility in accordance with the methods outlined by the U. S. Public Health Service for the testing of biological products. In addition, from each vial tested, plate 0.1 c.c. amounts of the vaccine on blood agar and pertussis vaccine medium, and observe daily for 7 days.

Antigenicity — Test the *agglutinability* of the vaccine with a Phase I antiserum which has an equivalent titer of at least 1:10,000. The vaccine should be agglutinated approximately to the titer of the serum. Test for *agglutinin production* in the rabbit by injecting 0.2 c.c., 0.4 c.c., and 0.8 c.c. respectively per kg. weight intravenously every 3rd or 4th day and testing on the 7th day after the last injection. If the equivalent titer is not at least 1:10,000 make a fourth injection of 0.8 c.c. per kg. rabbit weight and test again after 7 days.

Safety tests — Inoculate intraperitoneally 3 guinea pigs weighing about 300 gm. with 0.5 c.c., 1.0 c.c., and 1.5 c.c. respectively. Observe daily recording loss of weight and any symptoms of toxicity. All animals should live and should regain their original weight by the end of 2 weeks.

RELEASE OF VACCINE

Release a lot of vaccine only when tests of the final product show it to be sterile, pure, antigenic and safe.

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Methods in Health Education*

FOLLOWING the 1934 Annual Meeting, the *Chairman,* Marjorie Delavan, appointed a Committee on Methods in Health Education.

The work of the committee has been largely conducted by correspondence except for one meeting of 3 members at Saranac Lake in June, 1935, and conferences among members as opportunity permitted.

1. Consideration has been given to the objectives of the Section, of meetings, and of the health education movement. It is believed that an attempt should be made to formulate more fully definitions and perhaps criteria of standards of health education, and of relationships. In this regard, definitions prepared by the American Physical Education Association should be studied. (See December, 1934, *Journal of Health and Physical Education.*)

Contact should be established with the sub-committee of the Committee on Professional Education dealing with the problem of health training for health educators. (Sundwall, Shepard, Connolly, Edna Gerken, and Turner.)

2. From the viewpoint of our obligation to the health education movement, we may regard our meetings:

(a) As a place for presentation and discussion of tried (but not established) procedures and of established procedures, both as regards procedures in health education and in the fields of other sections which have a bearing on health education.

(b) As a place for expounding new conceptions which seem worthy of consideration. Ultimately there might be established a body of fact and experience for guiding principles and practices as a result of the accumulation of discussions over a period of years.

From the viewpoint of our obligation to the members, we might consider for meetings:

(a) Some definite didactic presentations under a leader who is a real teacher (the material in outline form to be given to the audience).

(b) Some forum and symposium meetings.

3. Consideration should be given to the desirability of preparing a manual of methods. Is it feasible to compile elementary methods in health education which would aim to be as specific as the directions for the standard methods of water analysis or the methods for record keeping (Walker and Randolph)? How inclusive can these methods be regarding health education in schools? Can a beginning be made in the formulation of a local publicity program for a health department? Could two or three subjects be selected for study the first year, with a referee appointed for each subject as was done by the laboratory section for the Committee on Standard Methods? For those who may argue that such crystallization of practice would result in stagnation, we need only point to the *Appraisal Forms* which have been revised from time to time as experience dictated.

* Progress Report of the Committee of the Public Health Education Section.

If the Section contains a large representation of health education teachers, a sub-committee from this group might formulate this phase of the Section program.

The Public Health Education Section should be the group to give positive and tangible suggestions for the promotion of health education in various types of communities.

More specifically, one member of the committee, Miss Nickerson, has summarized some of our obligations as a section as follows:

1. To professional workers

(a) Point out their potentialities in health education—facts that should be given to the public—newspaper publicity, how to detect a news story and write it up—use of statistics to show progress, etc.

(b) Place accepted technics and methods at their disposal, and assist them in making practical use of them.

(c) Teach them the right psychological approach so that groups in their community differing widely as to race, economic level, and intellect may receive health information in terms they can understand.

(d) Help them to coördinate their work with others toward a more complete health program in the community.

(e) Show them how to evaluate their results by the use of graphic methods.

(f) Help them to adapt their special technical information to practical use.

2. To schools

(a) Keep them well informed on authoritative scientific facts on health and disease.

(b) Point out special methods for presenting these.

(c) Place the best source material at their disposal.

To this end, our Section has a duty to its members

(a) To develop standards in health education.

(b) To put its stamp of approval on and present accepted technics and methods that are known to bring the best results.

(c) Teach them to look for results by way of graphic illustrations (it might be well to have a whole session on graphs and charts, how to make them, how to read them—their usefulness, etc.).

The committee consists of the following members of the Section: Homer N. Calver, Mary P. Connolly, Elizabeth Nickerson, Evart G. Routzahn, W. P. Shepard, and Marjorie Delavan, *ex-officio*.

IRA V. HISCOCK, *Chairman*

Bathing Places*

THIS committee has been asked to review the comprehensive reports of the former committee assigned to a study of this subject and present a report to both of our associations with a view to later acceptance by the Committee on Research and Standards of the American Public Health Association. The excellent pioneering reports prepared in 1925 and 1927 by the former committee headed by Stephen DeM. Gage have been used as a guide in swimming pool sanitation since they were published. In view of the completeness of these former reports, the present committee's report is to a considerable measure composed of the older reports. There are, however, some additions and modifications submitted, dealing largely with the following subjects: outdoor bathing places; cross-connections in swimming pools; streptococci tests; residual chlorine tests; foot infections; and bathing loads. Some of the discussion on these subjects is taken from the 1930 and 1932 reports of the Joint Committee. Where particular subjects are presented verbatim from the 1927 committee report (published in the February, 1928, issue of the *American Journal of Public Health*), the heading is marked with an asterisk. The same numbers are given to various sections as are in the 1927 report. This manner of presentation would seem to offer a more useful report than would a report whose reading is interpolated with comments on the older reports. It should be understood that the bulk of this report as to swimming pools represents the work of Mr. Gage, *Chairman,* and the other members of his former committees, to whom great credit should be given. No attempt has been made to revise the earlier reports except where changes were considered to be of importance.

Extent and Prevalence of Disease Transmission by Bathing Waters—This subject might be divided into two categories: those diseases which might be spread in relatively small swimming pools where the danger of transmission is from one bather to another, assuming a safe source of water supply, and those diseases which might be caused at outdoor bathing places where there are also the dangers from waters polluted by sewage from public sewerage systems and other extraneous sources. Another distinction might be drawn as to the types of possible diseases: whether eye, ear, nose and throat infections, or skin infections such as ringworm, eczema, scabies, etc., or venereal infections, or gastrointestinal disorders.

In 1921 a committee of the American Public Health Association attempted to assemble data on the subject of spread of disease from bathing waters by sending out 2,000 questionnaires to physicians and health officers, of which 627 were answered. The replies indicated suspicions that most of the diseases to which man is susceptible may have been caused by contaminated bathing water or insanitary bathing place appurtenances. Despite these replies, it must be admitted that there is a great

* Progress Report of the Joint Committee on Bathing Places of the Conference of State Sanitary Engineers and the Public Health Engineering Section of the American Public Health Association.

dearth of epidemiological information as to just what sickness is caused by bathing in polluted water.

Conditions pertaining to bathing waters are decidedly different from those surrounding public drinking water supplies. Many persons swallow little or no water during bathing—particularly is this true of salt water—and there is fairly rapid change of water at the majority of bathing places. These considerations tend to promote likelihood of occurrence of sporadic cases of such diseases as typhoid fever among bathers as against epidemics such as may occur with polluted drinking water supplies.

Considerable interest has been evidenced by many public health workers in the possibilities of spread of skin infections from bathing. Some cases of skin eruptions on the bodies of bathers have been reported. Some investigators have concluded that a skin infection designated as "Schistosome dermatitis" has affected bathers in the waters of lakes, rivers, ponds, or beach pools which support snails. The causative agent is said to be the *cercaria*, free swimming larval stage of certain parasitic worms, of the family *Schistosomatidae*. Unfortunately it is a fact that little is known as to the exact manner of spread of many skin infections—whether the infection is spread by the use of bathing place appurtenances such as walkways, common towels, suits, or drinking cups, or by other contacts such as might occur on a public playground, or whether the water has acted simply as a vehicle of transmission of disease germs from one bather to another, or whether the infection has been from bodily excretions present in the water.

Eye, ear, nose and throat infections are reported. Some of these might be due to lowering of resistance from chills; water may wash away protective mucous discharges containing antibodies, leaving the affected parts susceptible; harmful organisms already present in the body may be washed further into the ear and nasal passages during bathing; or excessive amounts of chemicals used for water purification may cause inflammation of mucous membrane and irritation of the skin. These are all possibilities aside from any harmful infections in the water.

The summary of the replies in the 1921 report when considered in the light of known epidemiological evidence, leaves this committee unconvinced that bathing places are a *major* public health problem even though bathing place sanitation, because of the health considerations involved, should be under careful surveillance of the public health authorities, and proper sanitary control of bathing places should be exercised. Indeed, this report is prepared for that particular purpose. It is realized that new epidemiological evidence may develop. It is agreed that common sense public health programs must recognize that bathing in polluted water is a potential danger, that insanitary conditions surrounding public bathing places are a hazard, and that common decency as well as health considerations dictate that reasonable steps should be taken to secure bathing in clean environments.

These conclusions are stated as an answer to a growing demand that this committee or some other group of public health workers propose a rigorous bacteriological or other standard, whereby bathing in certain outdoor bathing waters should be condemned from a public health standpoint. There is submitted in this report a proposed method of intelligent sanitary classification of outdoor bathing places with the inauguration of promotional educational programs to provide safer and more decent conditions as against extensive arbitrary programs of bathing place condemnation, and the recommenda-

tions of the 1927 committee toward provision of what are considered to be readily attainable and necessary sanitary precautions for swimming pools are also affirmed.

Drowning and Other Accidents at Bathing Places—This report includes various recommendations of the former committee as to safeguards against drowning and other accidents at bathing places, particularly at swimming pools. These safeguards should receive consideration from health workers.

I. *Classification of Bathing Places*
*A.
*B.

II. *General Principles of Bathing Place Sanitation*
*A.
*B.
*C.
*D.
*E.
*F.

III. *Bathing Beaches*

This subject is discussed in Sections XXIX to XXXIV of this report at considerably greater length than in the 1927 report.

SWIMMING POOLS

IV. *Location and Layout of Pools*
*A.
*B.
*C.
*D.
*E.

F. At indoor pools where chlorine disinfection is to be used it is recommended that the chlorine apparatus be so located as to be readily observed.

V. *Design and Construction Features*
*A.
*B.
*C.
*D.
*E.
*F.
*G.
*H.

I. Pool Lining. Including bottom and sides up to runways lining must be of white or light color material and present a smooth finished surface without cracks or joints. All corners must be rounded. Tile or glazed brick lining is recommended for all indoor pools and for small outdoor pools. White cement smoothly finished is satisfactory for large outdoor pools. Dirt does not show on asphalt or other similar dark material and such materials are not suitable for pool lining. Sand or earth bottoms cannot be kept clean and are not recommended for pools.
*J.

VI. *Proportioning Pool Area to Expected Load*
*A.
*B.
*C.
*D.

VII. *Inlets and Outlets*
*A.
*B.

C. Proper pipe connections must be provided in recirculation pools to permit water being drained to the sewer as well as to recirculation pumps. No direct connections to sewers should be permitted and all pool drains to sewers should be broken at a point where any sewage which may back up from the sewer will overflow to waste instead of being permitted to reach the pool. Pumping of pool drainage to an elevation above any possible sewer backing may in some cases be needed.

D. Inlets for fresh or re-purified water should be located to produce so far as possible uniform circulation of water throughout the entire pool. In semi-artificial pools of irregular shape a careful study should be made of probable circulation currents, and inlets should be located and spaced to provide as complete circulation as possible. Inlets from the circulation system should be submerged to reduce escape of chlorine odors. Where water from the public water system is added to the pool, cross-connections between the public water system and the swimming pool water should be eliminated by pumping make-up water from a pump suction well or admitting water to the pool above the overflow elevation of the pool. It is recognized that the avoidance of cross-connections in this manner may require the installation of a separate heater on the line supplying make-up water—particularly where large amounts of make-up water are added.
*E.

F. (Same as "G" in old report.)

G. (Same as "H" in old report except that words "cross-connections" in third sentence are changed to "connections.")

VIII. *Scum Gutters*
*A.
*B.
*C.

IX. *Steps, Ladders, and Step Holes*
*A.
*B.
*C.

X. *Runways or Sidewalks*
*A.
*B.
*C.
*D.

XI. **Visitors' Galleries*

XII. *Dressing Rooms*
*A.
*B.
*C.
*D.

E. All dressing rooms and appurtenances must be kept clean at all times. The use of an insecticide spray for lockers and of a disinfectant on floors, walls, and seats at frequent intervals is recommended. A 0.3 to 0.6 per cent solution of available chlorine is suggested as a disinfectant, to prevent the spread of foot infections. Foot tubs without proper disinfectant solution should be prohibited.

XIII. *Showers, Toilets, Lavatories*

A. Adequate shower bath facilities with hot and cold water must be provided at all artificial pools. The minimum number of showers provided should be in the proportion of one for each 40 bathers expected at time of maximum load, in the case of continuous bathing. For bathing by classes, as at schools, the number of showers may be taken as one-third the number of pupils in the maximum class.
*B.
*C.
*D.
*E.

F. Water flush toilets should be provided wherever possible. All toilets should be properly maintained.
*G.

H. The use of solutions containing 0.3 to 0.6 per cent of available chlorine has been found of value as a foot wash for the prevention of skin infections. At many swimming pools, bathers are required to rinse their feet in such a solution before entering the pools. Many authorities consider it preferable to place the foot bath at the exit from the showers to the dressing rooms so as to spread the chlorine over the dressing room floor and increase the time of contact. In some cases, 15 per cent sodium thiosulphate solutions have been used with success as a preventive foot wash but in one large city poor results with this chemical have been reported. One reason attributed to lack of success with sodium thiosulphate is failure to employ freshly made up solutions using a good grade of chemical. Sodium thiosulphate is a reducing agent and will reduce the available chlorine in the pool if bathers use it in a foot bath before entering the pool so that its use should be limited to bathers leaving the pool.

XIV. *Lighting, Ventilation and Heating*
*A.
*B.
*C.
*D.
*E.

F. All heating units shall be isolated or protected from contact with bathers to prevent injury. The heating units in dressing rooms, shower rooms, and toilets shall be capable of maintaining a temperature between 70° F. and 75° F. The pool room heating units shall be capable of maintaining a temperature between 75° F. and 82° F. Thermostatic control of the temperature is desirable.

G. The acoustical property of pool rooms has not received the attention it deserves. Designs and materials of construction, which will prevent reverberations of sound that result in confused noises, should be used. It is very important that an instructor's voice or a call for help may be clearly distinguished.

XV. *Recirculation System*
*A.
*B.
*C.
*D.
*E. (Add "below the water surface" after "Fixed pipe connections" in last sentence.)
*F. (Add "It is recommended that piping used for different purposes be painted distinguishing colors.")
*G.

H. Thermometers. At indoor pools a fixed thermometer shall be placed on the recirculation line beyond the heater and another near the outlet of the pool. At outdoor pools one thermometer is usually sufficient. Thermometers shall be accessible and have a type of scale that is easily interpreted.

I. Cross-connections. The avoidance of cross-connections on the pool piping system whereby pool water may under some conditions enter a potable water supply system which is connected either for admission of new water to the pool or for washing of filters is discussed in Sections VII D and XVII E. The possibility of polluting pool water from sewer connections is discussed in Section VII C. The dangers of cross-connections are also stressed under this heading.

XVI. *Proportioning the Water Interchange for Recirculation and Flowing Through Pools*

*A.

*B. (Change first two sentences to the following: "Gage and Bidwell have worked out the law of purification by consecutive dilution as applied to recirculation and flowing through pools and this is described by them as follows: ")

XVII. *Filtration*

*A.

*B.

*C. (Add after first sentence "Experience has shown that satisfactory results are secured when the effective size of the sand is about 0.4 to 0.5 mm. with a uniformity coefficient not exceeding 1.75. Sand should be washed free from clay, organic matter, and soluble material.")

*D.

E. Rapid filters of open gravity type must be equipped with loss of head gauges. Pressure filters must be equipped with pressure gauges, on both the inlet pipe and the outlet pipe for determination of loss of head or back pressure in the filter medium. Pressure filters should have a proper sight glass installed on the waste discharge pipe by which the operator may watch the progress of filter washing. Such glass should be readily removable for cleaning, and should be kept clean. When pressure filters are located at an elevation above the water line of the pool, each filter must be equipped with an automatic air relief valve. The arrangement and number of valves and interconnecting piping, or "valve nest," for necessary and convenient operation of rapid filters is fairly well standardized, and a discussion thereof may be omitted. It is usually desirable to have 3 or preferably 4 pressure filter units so that 1 unit can be cut out of use and washed with the filtered pool water. With 4 filter units in ordinary use, 1 filter can be washed with the use of the recirculation pump at 4 times the ordinary filtering rate. If washing with the recirculation pump is not feasible, a wash water pump of higher capacity may be installed and a suction well or small elevated feed tank, supplied from above with water from the public water system, can be used to supply water to the pump. Valved cross-connections whereby water from a potable water supply may be admitted directly to the recirculation system for the purpose of filter washing may permit pool water to gain access to the potable water supply because of leaking valves or suddenly lowered pressures. They shall not be permitted in the case of new swimming pools. In some instances, the elimination of existing cross-connections between potable water supply systems and swimming pools is difficult. Each case should be considered in the light of existing conditions, and remedies should be sought as dictated by the relative danger and the practicality of carrying out improvements. Where such cross-connections are permitted to remain in existing swimming pools, extraordinary precautions should be required to safeguard the potable water supply.

*F.

*G.

*H.

XVIII. *Disinfection*

A. From all available information, the addition of chlorine either as a gas or in a water solution by use of proper apparatus is today the most satisfactory method of pool disinfection. It is possible not only to disinfect the entire body of water in the pool completely with chlorine, but also maintain in the pool water at all times a residual amount of disinfectant to sterilize immediately any dangerous pollution disseminated by bathers. With the proper chlorine apparatus it is also possible to increase or diminish the dosage as required to compensate for variations in the bathing load. The committee recommends the use of chlorine either as a gas or in a water solution for disinfection of all pools where there is any appreciable bathing load, or where bathing suits are worn. Recent developments indicate that the use of ammonia and chlorine for swimming pool disinfection has become increasingly popular because of the more lasting qualities of the chloramines thus formed and the possibility of carrying higher disinfecting dosages without production of irritating effects from the use of large amounts of chlorine. Reports have reached the committee that much more satisfactory results from the standpoint of avoidance of loss of chlorine from disinfected swimming pool water and of less trouble with chlorine odors have been obtained by carrying high alkalinities in the water. Experiments indicate a delayed germicidal action of chlorine with high accompanying pH content of the swimming pool water, decidedly so at a pH of 7.7, similar to the delayed action with chloramine treatment, which is a factor to be considered. The fact that chloramines are decidedly slower-acting disinfectants than chlorine alone, makes it appear to the committee that chlorine is a safer disinfectant that chlorine and ammonia. Under some conditions such as in very large or outdoor pools, however, chloramine dis-

infection may produce better over-all results.

B. A possible objection to the use of chlorine for indoor pools is the chance of accidental escape of gas into the room. Modern chlorine apparatus is carefully designed and built to prevent possibility of such accidents. As a factor of safety it is advisable to install the chlorinator and chlorine containers in a special closet or room, with vents near the floor connecting with the outside air. A chlorine gas mask is a desirable safeguard to have on hand in case of accident. Such masks should be checked up occasionally to make certain that they have not decreased in effectiveness.

C. There are now on the market machines which feed hypochlorite solutions for disinfection. Chlorine compounds may be used to make up fairly stable disinfecting solutions and so long as their strength is checked by means of residual chlorine tests on the pool water, they may be very satisfactory. There are also chlorinators in use which produce chlorine by electrolytic action on sodium chloride solutions.

*D.
*E.
*F.
*G.

H. The use of ionized silver for swimming pool sterilization has been developed abroad and to a very limited extent in this country. While satisfactory bacteriological conditions have been reported in pools using such treatment, there is a question as to how rapidly this disinfectant acts on infectious material which may be discharged into the pool water by bathers. More information is needed concerning this process, pending which the committee is unable to make a definite recommendation at this time as to its use.

XIX. *Diving Towers, Spring Boards and Floats*

*A.
*B.
*C.
*D.

XX. *Emergency Equipment*

*A.
*B.

XXI. *Suits, Towels and Caps*

*A.
*B.
*C.
*D.
*E.
*F.
*G.

XXII. *Supervision of Bathers*

*A.
*B.
*C.
*D.
*E.
*F.

XXIII. *Personal Regulations*

*A.
*B
*C.
*D.
*E.
*F.
*G.
*H.
*I.
*J.

XXIV. *Chemical and Physical Quality of Swimming Pool Water*

A. Excess chlorine. Whenever chlorine, calcium hypochlorite, or other chlorine compounds, without the use of ammonia, are used for swimming pool disinfection, the amount of available or excess chlorine in the water at all times when the pool is in use shall not be less than 0.4 p.p.m. or more than 0.6 p.p.m. Whenever chlorine or chlorine compounds are used with ammonia, the amount of available or excess chloramine shall not be less than 0.7 p.p.m. or more than 1.0 p.p.m. Attention is directed to the possibility of interference by nitrites with the orthotolidin test, particularly when chlorine-ammonia disinfection is employed. If readings are made on the water to be tested within 5 to 10 minutes after the orthotolidin is added, and samples are kept away from the light during this period, the nitrite interference will be decidedly lessened. Standards for determining chlorine residuals shall be prepared and used according to the recommendations in *Standard Methods of Water Analysis* of the American Public Health Association. Standardized color discs and comparators may be used.

B. Acidity-Alkalinity. Whenever alum or sulphate of alumina is used during purification or repurification of swimming pool waters, the water at all times when pool is in use shall show an alkaline reaction. This means that the hydrogen ion content of the pool water shall not fall below 7.0.

*C.
*D. (Change 72° F. to 78° F.)

XXV. *Bacterial Quality of Swimming Pool Waters*

*A.
*B.
*C.

D. All chemical and bacterial analyses should be made in accordance with the pro-

cedures recommended in the *Standard Methods of Water Analysis* of the American Public Health Association in so far as these methods are applicable to swimming pool waters. In order to secure a true picture of the condition of the swimming pool water at the time of sampling, it is recommended that sodium thiosulphate be employed to neutralize the chlorine residual in the water sample bottle during transportation to the laboratory.

E. The part played by the various strains of streptococci in the respiratory diseases and their prevalence in the intestinal, buccal, and nasal discharges make the presence of streptococci in bathing waters very undesirable. Yet to eliminate them from swimming pools would mean decidedly smaller bathing loads and decided increases in chlorine residuals, either or both of which would hamper the usefulness of the pool. The committee calls attention to the fact that streptococci tests are of value in passing on the condition of swimming pool water but does not recommend any uniform standard limit for their presence.

F. 1. Preparation of bottle for sampling. All bottles of chlorinated swimming pool water shall be collected in bottles treated with sodium thiosulphate. The purpose of using water sample bottles containing sodium thiosulphate is to reduce the chlorine present in a treated water at the moment the sample is collected to prevent a continuance of the killing action of the chlorine on the bacteria while the sample is being transported to the laboratory. The bacteriological examination then shows the true sanitary quality of the water at the time the sample was collected.

2. Several procedures for preparing the bottles are presented.

For moist heat sterilization

Option 1. The sodium thiosulphate solution is prepared by dissolving 1.5 gm. of sodium thiosulphate in 100 ml. of distilled water. One-half ml. of this solution is placed in each clean bottle. (This amount has been found sufficient to reduce completely residual chlorine in an amount up to 2.0 p.p.m. in a sample of 130 ml. of water.) After the introduction of the sodium thiosulphate solution, the bottle is stoppered and capped. The bottles are then placed in an autoclave and sterilized for 15 minutes at a pressure of 20 lb. per sq. in.

Option 2. Into clean wet bottles, add approximately 0.02 to 0.05 gm. of powdered sodium thiosulphate. The amount need not be weighed. An estimated amount on the tip of a spatula is sufficiently accurate. The bottles are sterilized as in *Option 1.*

For dry heat sterilization

Into clean dry bottles is added from 0.02 to 0.05 gm. of powdered sodium thiosulphate as in A, *Option 2.* The bottles are stoppered, capped, and sterilized at 180° C. for 10 minutes. The temperature of sterilization must not approach 220° C. as sodium thiosulphate decomposes at this temperature.

G. Collection of samples. The samples should be collected by plunging the open bottle beneath the surface, sweeping the bottle forward until filled. The bottle should not be rinsed in the pool or the sodium thiosulphate will be removed. Samples should be collected only when the pool is in use and preferably during periods of heaviest bathing loads during the day.

XXVI. *Cleaning Pool*

*A.

*B.

XXVII. *Bathing Load Limits*

A. Frequency of Changing Water:

1. The total number of bathers using a recirculation type swimming pool during any bathing period shall not exceed 20 persons for each 1,000 gallons of clean water added to the pool during that period. The term "clean water" as used above may be interpreted to mean, new clean water used to refill the pool, new clean water used to replace loss by splashing or during cleaning, water taken from the pool and returned after effective filtration, or any combination of such waters. The bathing period shall be considered as the length of time the average bather stays in the pool. Under usual conditions this may be considered as 30 minutes. The pollution load in any swimming pool will not always vary in direct proportion to the number of bathers nor to the rate of change of the pool water, but will be influenced by effectiveness of filtration, types of bathing suits used, thoroughness of pre-cleansing baths, and other local conditions. Bacteriological examinations of the pool water should be the primary factor in deciding on bathing load limits for swimming pools. Under many conditions it may be found necessary to limit bathing loads still further—perhaps on a basis of applying a figure of 10 persons per 1,000 gallons of clean

water to the 30 minute bathing period as a maximum load in the pool at any one time. It is, of course, recognized that the daily turn-over ratio of pool water in recirculation type swimming pools is of importance as well as the rate of replacement of water during bathing periods, and a reasonable turn-over ratio is needed to help maintain satisfactory conditions in the pool. As stated under "Proportioning the Water Interchange for Recirculation and Flowing through Pools," a turn-over ratio of at least 2 and preferably 3 or more, for heavy bathing loads, should exist. The above figure of 20 bathers per 1,000 gallons in a 30 minute bathing period is offered on the basis of such a range of turn-over ratio. It is the opinion of the committee that with a properly maintained pool the maximum load of bathers in the pool at any one time is of more importance in arriving at safe bacteriological conditions than the total number of bathers per day although intervals when no bathers are in the pool undoubtedly aid by promoting almost complete intermittent sterilization.

2. The total number of bathers using a fill and draw swimming pool shall not exceed 1 person for each 500 gallons of water in the pool between complete changes of pool water without disinfection. Where intermittent disinfection is employed, the number of bathers using the pool will be governed by the safe limits mentioned under "Proportioning Pool Area to Expected Load," and by bacteriological analyses of the pool water. The committee does not recommend the use of fill and draw swimming pools, as stated elsewhere.

3. The total number of bathers using an outdoor pool—partly artificial and partly natural in character, dependent for circulation and replenishment upon an inflow and outflow of water from a clean stream or a well, spring or piped water supply—might be based upon the computation outlined under (1) as a maximum limit, although safe bacteriological conditions should be the principal guide. Where pre-cleansing baths are not used at such pools, reductions in bathing loads will undoubtedly be found necessary to maintain safe bacteriological conditions even with high chlorine residuals. Pre-cleansing baths should, of course, be provided at all pools.

B. Frequency of Disinfection. The committee has decided to omit a previous recommendation as to bathing load limits between successive disinfections in pools practising intermittent chlorine disinfection in view of the recommendations as to maintenance of adequate chlorine residual *at all times* the pool is in use. It is pointed out that experience indicates that far better bacteriological conditions can be maintained with continuous chlorine disinfection. Where a pool is operated with wide fluctuation in chlorine residuals, chlorine resistant organisms may develop at times when the chlorine residual content of the water is low, and it may be necessary to use very high chlorine residuals to destroy them.

C. Area Limitation. One investigator (Mallmann) has concluded that under normal pool recirculation and design conditions, a maximum bathing load limit of 1 bather per 35 to 45 sq. ft. of pool area will result in safe bacteriological conditions with adequate chlorine residuals. In the light of experience, these figures seem reasonable. This area per bather is greater than provided for under the design requirements set under "Proportioning Pool Area to Expected Load," Section VI.

XXVIII. *Operating Control*

*A.

*B. (Change last sentence to read: "Tests for excess chlorine in the water shall be made as frequently during the day as experience proves to be necessary to maintain adequate residuals.")

C. Tests for Acidity. At any pool where alum or sulphate of alumina is used or where artificial alkalinity is added to the water, the pool operator must be equipped with a hydrogen ion testing outfit and must take hydrogen ion tests on the water every day the pool is in use, and more often if necessary.

D. Operating Records. Every pool operator must be supplied with a proper notebook or with blank forms on which shall be recorded every day the number of persons using the pool, peak bathing loads handled, the volume of new water added, the temperature of the water, and the temperature of the air. Whenever a pool is used by both males and females the number of each and whether adults or children should also be recorded. At all pools where artificial circulation, filtration, or any chemical treatment is used, a full daily record must also be kept of the actual time pumps and filters are in operation, of the time each filter is washed or cleaned, of the time and amount of each chemical used or added, of the time the bottom and sides of the pool are cleaned, and the results of all hydrogen ion, excess chlorine, or other tests.

OUTDOOR BATHING PLACES

XXIX. *Definition*

Under this heading are considered bathing places along small streams, rivers, lakes, and tidal waters. Fill and draw and recirculation bathing pools readily subject to artificial purification or to constant replenishment with uncontaminated water are not included.

XXX. *Sources of Pollution*

In a swimming pool whose water is derived from a public or other supply of unquestioned quality, it may be assumed that the presence of organisms of the coli-aerogenes group indicates pollution by human sewage particles. The presence of such bacteria in outdoor bathing places, however, may be due to the wash from cultivated fields, animals, and generally harmless contamination. Routine bacteriological tests do not differentiate between harmful and harmless contamination in such cases. Harmful contamination may be caused by sewage from boats, individual dwellings, hotels, factories, or other establishments; public sewerage systems; refuse dumping; and bathers themselves.

XXXI. *Flowing-through Bathing Pools along Small Streams*

The use of small natural or dammed-up pools along small streams by large numbers of bathers is not recommended unless disinfection is provided, as discussed in the following section. Where such pools are proposed to be used, and will be dependent upon the natural stream flow for cleansing and dilution, it should be ascertained that there is a constant and appreciable overflow of water past the dam under all weather conditions when the pool is to be used. Any small pool patronized by a number of bathers is certain to show bacteriological pollution in considerable amounts unless disinfection is provided. While no specific amount of diluting water for such pools can be recommended, it is probably fair to say that less than 500 gallons per bather per day is too small a diluting volume without disinfection.

XXXII. *Disinfection of Small Flowing-through Bathing Pools*

Disinfection is desirable to counteract pollution introduced by bathers. Hypochlorite in solution or in powdered form may be added to the pool inlet or at various points over the pool area. Chlorination of the pool inlet may be continuous. Several applications of disinfectant over the pool area during the bathing period are usually preferable to one application. Even with disinfection, the same governing factors should be considered in arriving at maximum bathing loads in small outdoor pools as presented under "Swimming Pools" (Sections VI and XXVII), and also the same limits for chlorine residuals are recommended as for swimming pools. Disinfection of large bodies of water is discussed in the following section.

XXXIII. *Disinfection of Large Bodies of Water*

The disinfection of relatively large bodies of water by use of a chloro-boat, so called, has been used with apparently some success in a few scattered instances. In some locations, bathing areas several acres in area have been disinfected satisfactorily by the use of extensive piping systems along the water bottom either for distribution of chlorine disinfecting solutions or for distribution of large amounts of pumped water drawn from the bathing area and disinfected in the pump suction with chlorine or chlorine and ammonia in what is practically a recirculation system. Where such disinfection is feasible, the same contents of chlorine and chloramine residuals are recommended as have been proposed for swimming pools. Chlorine and ammonia are undoubtedly more practical of application for large outdoor bathing areas than chlorine alone due to the greater persistence of the chloramines in the water. This probably outweighs the disadvantage of the slower disinfecting action of the chloramines as compared with chlorine. While the possibilities of disinfection methods deserve consideration, unless the future brings forth further developments in the way of attempt to establish bacteriological standards for outdoor bathing places, it appears that emphasis will be laid on the reduction of pollution of outdoor bathing areas rather than on attempt to counteract such pollution by disinfection of bathing waters. The use of disinfecting agencies, however, may be developed particularly to guard against dangers from pollution by bathers themselves in those densely populated bathing areas which are not subject to major water changes through the action of tides and currents.

XXXIV. *Collection of Samples from Outdoor Bathing Places*

Analyses of samples of bathing waters intelligently interpreted are of great value but full consideration should be given to the conditions under which samples are collected and the conditions which may exist at other times. The replenishment of bathing water by stream flow, by tidal action, and by wind and temperature currents, the contamination

introduced by bathers themselves and the intermittency of various sources of sewage pollution, are all of importance. In considering dangers from sewage pollution of bathing areas, it is well to emphasize that time is a factor of great importance. The hazard from a relatively small amount of sewage pollution in close proximity to a bathing area is far greater than a large amount at a considerable distance.

XXXV. *Bacteriological Classification of Bathing Waters*

In the collection of samples from bathing waters in connection with a scheme of relative classification of bathing waters which is subsequently presented, it is suggested that in arriving at a coli-aerogenes count on samples, a simple procedure is to run 4 or 5 dilutions from 10 c.c. down on each sample, in accordance with the expected amount of pollution and to assume 1 *B. coli* originally present in the greatest dilution to give a positive test. For example, positive in 10 c.c. and 1 c.c. and negative in 0.1 and 0.01 c.c. would be called 100 *B. coli* per 100 c.c. Where so-called anomalous results are occasionally obtained such as a sample showing positive in 10 c.c., negative in 1 c.c., and positive in 0.1 c.c., it is suggested that the greatest dilution result be recessed to the next, which in the case illustrated would give 100 *B. coli* per 100 c.c. This is an arbitrary method of computation, open to some mathematical objections, but it is simple and is satisfactory for all practical purposes. On the basis of experience, where a large number of samples has been handled, presumptive tests on lactose broth may be considered sufficient evidence of the presence of *B. coli*, the resulting error as against complete confirmation according to *Standard Methods of Water Analysis* being slight.

XXXVI. *Relative Classification of Bathing Areas Recommended*

In passing on waters of outdoor bathing places, three aids are available: (1) the results of chemical analyses of the water; (2) the results of bacteriological analyses of the water; and (3) information obtained by a sanitary survey of sources of pollution, flow currents, etc. Chemical analyses may in some cases be of value but are not ordinarily delicate enough tests.

It is not considered practicable or desirable to recommend any absolute standard of safety for the waters of outdoor bathing places on any of the three above bases. The arbitrary wholesale condemnation of bathing beaches representing large capital investments is unwarranted without definite epidemiological evidence. A relative scheme of classification of outdoor bathing places appears to offer the most promising program for public health workers to follow. Due to the difference in local conditions surrounding bathing in tidal waters, large and small lakes, and large and small streams, the degrees of classification of the bathing waters in any particular region may of necessity be varied.

In one state, a classification survey of the shore waters was carried out. The entire shore line was divided into sampling stations about 1,000 ft. apart and samples for bacteriological analysis were collected in from 2 to 6 ft. of water at high, low, half ebb, and half flood tides at each station. The results were averaged and adjoining stations combined into sections on the basis of the sanitary survey information. The analytical classification for each section was made by averaging the averages for the included stations. The sections were then classified on the basis of the analyses as follows:

	Average B. Coli per 100 c.c.
Class A	0– 50
Class B	51– 500
Class C	501–1,000
Class D	over 1,000

These same sections were also classified in Classes A, B, C, and D on the basis of sanitary survey information as to sewer outlets, float studies, etc., and final classifications were based on both the analysis and sanitary survey classifications. In the sanitary survey classifications, Class A was considered good; Class D was very poor; and the two intermediate classes ranged from doubtful to poor. Close correlation was obtained between the analysis and sanitary survey classifications.

Proposed bacteriological standards by various agencies have seemed to hit mainly upon two widely divergent limits for standards of acceptability for bathing waters, one of which is 50 *B. coli* per 100 c.c. and the other of which is 1,000 *B. coli* per 100 c.c. It is perhaps reasonable to conclude that, subject to interpretation of analytical studies from proper angles, waters better than the lower limit (1,000 *B. coli* per 100 c.c.) are fairly acceptable. Both these lines of demarcation are drawn in the classification scheme just presented and the committee recommends the use of this classification scheme unless local conditions make some other classification scheme preferable. If it is desired to set up any intermediate classifications, any classification such as "A" can be broken up

into "A+" and "A—". The interpretation of areas falling into Classes B, C, and D as to whether these areas can be considered good, doubtful, poor, or very poor, must for the present be left with the interested state health department or other agency concerned. As further information is gained from the classification of areas, more definite conclusions may be reached. It is emphasized again that final classifications should not be made upon the basis of bacteriological analyses alone but should depend largely on correlative sanitary survey information. Allowances should be made and distinctions drawn as to pollution introduced by large bathing loads at outdoor bathing places and pollution derived from sewer discharges or other sources.

The committee feels that the health board or department of each state should be the guiding agency for state-wide studies of outdoor bathing areas, with the assistance of community and district health units where practicable. Information obtained as a result of classification surveys of bathing waters should be in the hands of public health workers to enable them to furnish intelligent answers to inquiries on the part of the public. While data of this type should be released advisedly so as not to cause unwarranted depreciation of property values near bathing areas, it should serve to acquaint the public with danger spots caused by uncontrolled discharge of sewage and to promote the betterment of health conditions by installation of sewage treatment where necessary for the protection of bathing waters. The recent studies and recommendations of the Tri-State Pollution Commission (New York, New Jersey, and Connecticut) point out what may be done in the way of specifying degrees of treatment for discharge of sewage into waters allocated for recreational purposes.

XXXVII. *Sanitary Appurtenances at Outdoor Bathing Places*

Attention is directed to the need for proper sanitary appurtenances at outdoor bathing places. The remarks in Sections XII and XIII under "Swimming Pools" with regard to dressing rooms, showers, toilets, and lavatory accommodations, are also pertinent as to outdoor bathing places.

NOTE: This 1935 report of the Joint Committee on Bathing Places is presented with the hope that the committee may be advised as to any proposals for alterations and additions to the report. Any such proposals will be given careful consideration and it is intended to await reception of such proposals before recommending the acceptance of the report by the Committee on Research and Standards of the American Public Health Association.

WARREN J. SCOTT, *Chairman*

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PROPOSED STANDARDS *

Bacterial Quality of Swimming Pool Waters

A. Determination of Bacteria Counts.

1. The medium used shall be nutrient agar, prepared according to *The Standard Methods of Water Analysis* of the A.P.H.A.

2. The bacteria count shall be made from plates that have been incubated at 37° C. for 24 hours.

3. Standards. Not more than 10 per cent of the samples covering any considerable period shall contain more than 200 bacteria per c.c.

B. Determination of the Colon-Aerogenes Groups.

* This report was prepared through the collaboration of the Michigan and Detroit Departments of Health and Michigan State College.

1. The method of procedure and media shall be those recommended in the *Standard Methods of Water Analysis* of the A.P.H.A.

2. The standard portion of water for this test shall be 10 c.c. and the standard sample shall consist of five standard portions of 10 c.c. each.

3. All fermentation tubes showing 10 per cent gas shall be confirmed for the coli-aerogenes group by smearing eosin-methylene blue agar plates or transplanting into 2 per cent bile brilliant green broth. Refermentation tests may be made from the eosin-methylene blue agar plates at the discretion of the analyst.

4. Standards. The average colon index for 10 or more consecutive samples shall not exceed one per 100 c.c. of water.

At least 2 samples for bacteriological test should be taken each week.

The residual chlorine test shall be made at the pool side at the time the sample is collected.

All samples shall be tested at the laboratory at the time of bacteriological analysis for residual chlorine as a check against the removal of the chlorine by the sodium thiosulphate at the time of collecting the sample.

The following facts are presented in support of the above recommendations:

Since January 1, 1933, all swimming pool water samples in Detroit have been collected in bottles containing sodium thiosulphate. A total of 11,715 such samples have been examined through August of this year. Of the 11,598 plates examined (37° C. in 24 hours on agar), 749, or 6.5 per cent, had total bacterial counts in excess of 100 per c.c., 618, or 5.3 per cent, were in excess of 200 per c.c. and 316, or 2.7 per cent, were in excess of 1,000 per c.c. Of the 58,575, 10 c.c. portions examined for *E. coli* (partially confirmed by refermentation in 2 per cent bile brilliant green broth), 1,463, or 2.5 per cent, were positive. The average index of *E. coli* was 0.25 per 100 c.c.

Factors Affecting Work Program of Municipal Public Health Engineers*

PUBLIC health officials have never before had the opportunity of obtaining for their communities the valuable and permanent benefits which now are, or may be, made available through the employment of persons on the work relief and social security programs. Where ordinarily the opportunity for study, correlation of data, and research is limited by local budgets and number of employees, there is now available as part of the federal work relief program ample funds and in many cases personnel. Not all of these benefits will come from the extension of present health departmental activities, but may accrue by the encouragement of undertakings by other governmental agencies in their respective fields. The relationship of these various activities either directly or indirectly to the conservation of public health is recognized and appreciated by the trained sanitarian, and he is therefore the logical person to act as a coördinator for the promulgation of such a program.

The public health engineer not only has an unusual opportunity to serve his community directly, but he shares a civic responsibility to see that work relief funds are expended upon projects of maximum benefit. Much valuable data are gathering dust in the archives of our health departments and other governmental agencies because of the lack of local funds and personnel to assemble and correlate them. The construction of sanitary facilities has been curtailed to a minimum because of limited budgets. There exists today an unusual opportunity for developing programs of these types which will be of permanent benefit to public health.

As an aid to public health engineers and a guide to health departments that do not have the services of an engineer, the following list of possible projects is suggested. It is understood that the health departments may not have jurisdiction over some of the activities mentioned, but it is hoped that through their influence and guidance they will be able to encourage other governmental agencies along the lines of endeavor that will bring about permanent improvements in environmental sanitation and thereby create an improvement in the public health.

Construction programs for the extension of water mains and sewers, the erection of water or sewage treatment plants, and the rehabilitation and improvement of public hospital buildings and equipment are examples of major types of improvements that will give permanent health benefits to any community. Such programs may depend upon the ability of the locality to participate in their financing. There are, however, a large number of projects which can be carried on as a part of the work relief program.

The suggested list follows: Surveys embracing such subjects as environmental sanitation, housing, industrial hygiene, faulty plumbing, noise abate-

* Progress Report of the Committee on Municipal Public Health Engineering.

ment, ventilation, air pollution, milk sanitation, bathing facilities, stream pollution, land use surveys, city planning, and provision of recreational facilities, can be conducted. Active campaigns for rat eradication, cutting and pulling of weeds, cleaning of vacant property, mosquito control, adequate dish washing and sterilization, and the demolition of buildings which are unfit for human habitation can be carried on. The assembling and correlating of data relative to nuisances, water supplies, sewers, municipal wastes, and other environmental factors can be undertaken.

In planning any type of program the sponsor is cautioned to check carefully with his local work relief agencies in order to determine that the needed personnel will be available. A program based upon the advance information of the kinds and types of personnel available is more likely to succeed than one in which an attempt is made to adapt an unsuited group to the project.

The committee believes that if these precautions are observed and the work is carried out under the direction of a competent public health engineer, lasting contributions of great value to the health of our people will be achieved through the use of facilities now available.

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Public Health Aspects of Noise Abatement*

RECENT developments in the abatement of urban noise indicate that the movement is based upon the reaction of the general public to noise as a nuisance and to the desire on the part of municipal officials to abate this nuisance. Generally speaking, municipal health departments have not been active in this field, although the public usually registers complaints as to noise or other nuisances with health departments, irrespective of the provisions of local ordinances. Although some publicity campaigns have resulted from the activities of municipal health officials, nevertheless, organized noise abatement campaigns in Europe and this country are usually under the direction of noise abatement commissions especially appointed for the purpose, because of the realization by municipal authorities that noise abatement has many aspects requiring the coördination of the activities of several municipal departments. This is evident when the sources of urban noise are considered.

Noise may be classified in two groups from the standpoint of administrative procedure, namely, the noises unavoidable at present, and those noises which can be controlled or eliminated. The unavoidable noises would be the residual noises remaining even with properly controlled traffic of modern vehicles in satisfactory state of repair, the use of modern building methods, noise hoods or screens around noisy machinery, etc. Any appreciable reduction in this type of noise will have to wait upon advances in engineering design and construction, and the replacement of obsolete noisy equipment when worn out. That such advances are in progress is evidenced by the marked improvement in the modern automobile, which is surprisingly quiet when one considers the power involved. Furthermore, future developments are to be anticipated because of the active interest shown in the reduction of noise by engineers and architects stimulated by the public demand for quiet machines. In fact, the National Standards Association, representing a number of technical organizations, is coördinating the efforts of a number of committees on noise. The present activities of these committees pertain to fundamentals of acoustics, analysis of origin and magnitude of noise, and method of preventing or dampening noise. The practical results, therefore, will be the development of more quiet machinery and sound absorbing building materials, which may be used in general construction to reduce noise to a minimum.

Noises of the second group, namely those which can be avoided by an intelligent and active campaign in noise abatement, have been shown by careful investigation to emanate primarily from traffic sources, especially the operation of elevated railways, street cars, automobiles and trucks in a poor state of repair, and the unnecessary use of the automobile horn and muffler cut-outs. On the other hand, many complaints are received as to the noise from the use of radios late at night, the delivery of milk in the early morning hours, and the noisy collection of ashes and garbage. Construction activities are shown to be of minor

* Report of the Committee on Noise.

importance to a municipality as a whole because of the localized nature of noises resulting from building operations, such as riveting. It is gratifying to know that the development of welding processes is likely to supersede riveting and remove this objectionable source of noise from municipalities. Probably the most important advance in the construction field, however, is in the use of sound absorbing materials to dampen unavoidable noise.

The general public is not much concerned with noise as a menace to its health, but merely as a nuisance which should be abated. A number of individuals agree with many members of the medical profession in viewing with alarm the interference of noise with sleep, and the resulting ill effects of nervous strain. The general public, therefore, will support campaigns for noise abatement whether conducted by a noise abatement commission, police departments, or municipal health departments, provided publicity precedes such campaigns.

Generally speaking, municipal health officials are not interested in the abatement of noise because of their absorption in more important public health activities, and because there are no definite data indicating the effect of urban noise upon the mortality and morbidity rates of municipalities. Furthermore, the conclusions of eminent neurologists and physiologists as to the harmful effects of noise, and the fact that there are more than 25 noisy trades where deafness is a common occurrence among the workers, apparently has not convinced many municipal health departments that noise abatement is their immediate concern. Hence the general attitude seems to be that noise abatement is a broad municipal problem with many aspects other than the protection of public health.

Existing ordinances and laws in most municipalities provide administrative procedure for the abatement of noise, provided whole-hearted support is given to the enforcement of such ordinances. Studies by the Noise Abatement Commission of New York City have indicated that the multitude of miscellaneous ordinances of any given municipality are so scattered that administration is very difficult. Furthermore, another stumbling-block in the administration of most of the existing ordinances is that they specify that those producing avoidable and prohibited noises are committing a misdemeanor which is punishable by fines of $100 to $500, or imprisonment of 6 to 12 months. This severe penalty handicaps police officers in the enforcement of petty violations of noise abatement ordinances which, nevertheless, constitute the chief source of urban noise when considered collectively. The New York City Noise Abatement Commission has recommended that the city charter be revised to permit the imposition of so-called administrative fines of $5, and similar action might be warranted in other municipalities.

The procedure under an ordinance permitting the imposition of administrative fines would be for the violator to be handed a notice of his violation by the police officer, sanitary inspector, etc., which would indicate that the violator must pay a $5 fine at the office of the collector of taxes, rather than at a police court, except in those cases when the violator protests his innocence and demands a police court hearing, in which case the usual procedure would be followed. It may be concluded from this very careful study of local procedure required in the abatement of urban noise in a large municipality, that special noise abatement ordinances will greatly facilitate the control of this problem provided the city charter permits of the procedure outlined above.

Little can be accomplished in the

abatement of urban noise unless the general public is educated in the needlessness of most noise, its ill effects, and the possibility of its abatement. It is evident, therefore, that educational campaigns should precede any serious attempt at the control of this problem. Probably the most effective campaign is to acquaint the public through the press and radio as to the effect of noise upon the general well-being of the urban population and the results of investigations as to the origin and magnitude of noise from specified sources, and as to the proposed comprehensive campaign in noise reduction, such as that in progress in New York City, and elsewhere. In this way the coöperation of the public can be secured, and the necessary information obtained as a guide to the progress of the abatement campaign.

A widespread publicity campaign of this nature, and the organization of administrative machinery employing police officers, building inspectors, sanitary inspectors, etc., can be accomplished only through the organization of noise abatement commissions or committees, with active executives to supervise the work. It appears to this committee that sanitary engineers employed by municipal health departments are especially fitted to coöperate actively with noise abatement commissions, and to serve in many cases as the executive officer. In this way the support and influence of municipal health departments can be secured without placing undue stress upon the public health aspects of noise abatement.

The committee recommends, therefore, that those interested in the abatement of urban noise proceed along broad administrative lines through the formation of noise abatement commissions. Due weight must be given to the necessity of active publicity campaigns and the correlation of the activities of many municipal departments and bureaus so that the available administrative personnel will be utilized. Unless this is done the activity will be merely the passing of a general ordinance for lackadaisical enforcement by police departments, after the original interest has waned.

It is felt that the stimulus of an active health department is required to organize such activities and to lend assistance through active coöperation on the part of municipal sanitary engineers and sanitary inspectors. In fact, municipal health departments may have to assume more detailed supervision of such activities in the smaller city through working in close coöperation with police departments. The sanitary engineer in such cases should supervise the collection of technical data and develop control procedures to be followed by the police and other municipal employees. In this way this phase of the control of the environment can be assigned its rightful place with other environmental factors subject to control by those engaged in the field of sanitation and public health.

We respectfully submit this as a final report of the committee, inasmuch as original research by the committee is not possible, thus limiting its endeavor to the appraisal of current developments. It may be anticipated, however, that a critical review of the subject at some future date may be warranted, after the practical aspects of noise abatement have justified definite administrative procedures and have indicated the place of noise abatement in the public health program.

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Recent Developments in Plumbing as Related to Public Health*

OF several recent developments in the field of plumbing, the movement to investigate the health hazards in plumbing of all federal buildings appears to be one of the most significant. Another is the increasing demand for some nationally recognized authority to pass upon the merits of various plumbing fixtures from the health standpoint. The effort by plumbers to inform themselves and the public more fully, as to what is safe in design and practice, has gained momentum and greater scope, aided by their national, state, and local associations. Manufacturers have continued to improve certain fixtures, eliminating features which menace health, and to develop additional protective devices.

In the last United States Congress, Representative John D. Dingell of the 15th Michigan district introduced in the House, and Senator Royal S. Copeland of New York sponsored in the Senate, a joint resolution calling for a survey of the plumbing in all federal buildings to discover defects that might cause disease outbreaks. The resolution was passed by Congress. It now appears probable that the survey will be made under the supervision of the U. S. Public Health Service, with the aid of the Works Project Administration, starting in two cities to develop methods and determine the practicability of extending the survey to all federal buildings. This work will safeguard the health of federal employees and of the public visiting federal buildings, and set an example for owners of other buildings.

Much of the interest attaching to this survey arises from the fact that Mr. Dingell has indicated his intention to follow through, and do everything possible to assure that plumbing health hazards, when discovered in the study, will be promptly removed, and that adequate plan examination before, and supervision during, construction will be provided to prevent new hazards in government buildings.

In the correction of plumbing defects which are hazardous to health, more specific information appears to be needed upon the adequacy and dependability of various devices. This demand has been felt in several quarters and was given expression in a resolution passed at the 1935 annual meeting of the National Association of Master Plumbers.

Some thoroughly competent scientific body having national recognition among public health authorities should secure and make available the pertinent facts, and make recommendations so that a building owner, health officer, or plumber on the job will have something more than his own judgment to guide him. Many decisions relating to plumbing devices have been made by local authorities, to meet the demand for something with which to go ahead. Uniformity would be promoted and confusion minimized by a national set of standards acceptable to all concerned.

During the past year, the public health importance of plumbing has been brought before the industry as perhaps never before. The 1935 convention of the National Association of Master

* Progress Report of Joint Committee on Plumbing of the Conference of State Sanitary Engineers and the American Public Health Association.

Plumbers last June featured health in an outstanding way upon its program and in the exhibits. Special demonstrations of water contamination by sewage through faulty plumbing were given to groups of master plumbers from all parts of the country. These were made possible through the cooperation of the local Master Plumbers Association, the National Association, the Journeymen Plumbers Union, the manufacturers of plumbing supplies, Mayor Edward J. Kelly, Dr. Herman N. Bundesen, and other city officials of Chicago, where the convention was held. The exhibit of the Wisconsin State Board of Health here has a similar objective. The National Association of Master Plumbers has formed a Public Health Committee upon which the American Public Health Association and other organizations are represented.

Numerous state and local plumbing groups have given particular attention to the subject of water contamination, both at their meetings and in their publications, in order to spread information and stimulate interest. Likewise, the American Society of Tropical Medicine, the Pan-American Medical Association, the Conference of State and Provincial Health Authorities of North America, the American Water Works Association, the American Hospital Association, the Western Branch of the American Public Health Association, and the American Society of Sanitary Engineering, in addition to a number of state and local groups, have given some time on their programs to this subject within the past year. At the American Hospital Association meeting, the members were privileged to witness demonstrations of water contamination caused by plumbing defects through the courtesy of the Board of Education, Department of Health, and Master Plumbers Association of St. Louis.

Experimental plumbing work in many fields by universities, technical colleges, manufacturers, and health departments, or others in charge of plumbing inspection, has been continued with material contributions to the fund of knowledge. Some work relates to health and other studies to water waste, water hammer, corrosion, etc. Unfortunately, the data accumulated have in some instances not been widely broadcast as yet, though in some other cases, such as Wisconsin, valuable bulletins or articles in the trade press have appeared. Such work deserves every encouragement by public health engineers. The Plumbing and Heating Industries Bureau has aided in securing publication of important items on health factors in plumbing, especially in trade and daily papers, and in arranging radio broadcasts on this subject.

There can be reported at this time a growing recognition that it is folly to permit contamination of drinking water in a plumbing system after spending huge sums to prevent pollution at the source or to purify the water. The wide variety of uses for water in any large city, and the complexity of plumbing systems in buildings of considerable size make the safeguarding of drinking water against bacterial and protozoal infections and chemical poisons no easy task. The recent progress and the present development of widespread interest are most encouraging for the future. It is important that the further activities be properly directed, if they are to achieve their maximum usefulness. This necessitates well qualified men who possess not only technical knowledge, but plenty of good common sense.

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Special Problems in Park Sanitation*

THE growing interest in public park areas to preserve points of natural scenic beauty and historic interest, and to furnish camp and picnic grounds for recreational use, has introduced new phases into the familiar problem of camp sanitation. The questions of sanitary practice are much the same as those encountered in tourist camps, but there is a definite difference in administrative control. The extent of the development of this type of recreational areas is evident when you consider the National Parks—51 national monuments, and the 450 state parks that have been created. The national parks extend east and west, north and south over the country, while New York and Texas head the list of state parks with 32 each; Illinois has 30; California 29; and Massachusetts has 24. The other states range from 16 parks down to a single one, while only 3 states—Delaware, Kansas, and Louisiana—do not have any.

Apparently there is need for a general policy to direct the sanitation of recreational environment. A few of the essential points that should be considered in formulating such a policy are suggested by the sub-committee in this brief preliminary report. Glancing back a few years to the early stages of general transcontinental motor vehicle travel, the free tourist camps are called to mind. Every municipality with any pretensions had one. You may remember the insanitary chaos of these vacant lot, free camps, that were established to lure the traveller to spend a few hours and dollars in roadside towns. The lessons learned at that time can well be used in formulating a policy for state and county parks.

The development of each new state or local recreational area imposes an obligation for additional paid service by the governmental body in charge. Parks of this type when established should make adequate provisions for supervision by a paid caretaker whose services will be continuous during the portions of the year the area is in use. The public when at play can scarcely be expected to have more orderly habits than when touring. The continuing success of these parks will largely depend upon their being maintained with sufficient care so that the natural charm is not marred by unsightly collections of debris. The vacationists must continue to have a feeling of unspoiled country.

In the early stages of a movement, communities are apt to be carried away with an idea, losing sight of its practical aspects. Camps in public parks may furnish pleasure for a large number of people of all ages, but they will be enjoyed only so long as they are well maintained. The number of such camps in any area is limited by the state's ability to furnish adequate supervision. Only camps should be permitted that are absolutely necessary to care for the usual holiday crowds, and new ones should not be established until there is a definite public demand.

Organization for maintenance could be modeled on the National Park Service. This may, however, be too elaborate for the less populous states. The

* Preliminary Report of the Sub-Committee on Promotion of Environmental Sanitation.

sanitation of parks and recreational areas should be planned and supervised by the engineering division of the state board of health. The actual construction work and maintenance should be under the jurisdiction of the state park board, if one exists. It may be possible in some instances to have the highway department supervise construction and maintenance. Certainly some established department must be charged with this responsibility. It is difficult to imagine how parks can long exist in active use that are not controlled by a department directly charged with their care. Concessions are a feature of many parks, and in some instances the concessionaire might be required to act as caretaker under the direction of a governmental department.

These concessions may be anything from a hot dog stand at the entrance, to a luxurious hotel, but all of them dispensing food should be regularly inspected.

A uniformity of design for structures planned is desirable, the exteriors being in keeping with the surrounding country. Sewage and garbage disposal plants must not only be planned to afford the usual protection, but they must also be inconspicuous; located where they will mar neither the view nor the pine scented air.

The Civilian Conservation Corps, the Public Works Administration, and the Federal Relief Administration have done such splendid work in developing large park and recreational areas, that it now behooves us to formulate a general policy to govern the sanitation of these areas so that the health of the people using them will be safeguarded. A few of the salient points that may be included in such regulations are listed below:

1. Do not establish a state or local park, recreational area, or camp site without providing for a caretaker.
2. Do not have more camps than are absolutely necessary to care for the usual holiday crowds. Camps should not be established until there is a definite public demand.
3. A small charge should be made to all patrons using camp grounds.
4. Each new development imposes an obligation for additional paid service.
5. There must be an adequate supply of safe, palatable water, provisions for garbage disposal, and conveniently located comfort stations.
6. Concessions should be inspected to guarantee that they are complying with sanitary regulations.
7. Swimming pools and bathing places should comply with the American Public Health Association regulations; and should have a lifeguard on duty.
8. Sanitation should be planned and supervised by the engineering division of the state board of health.
9. Construction work and maintenance should be under the jurisdiction of the state park board or a designated governmental department.

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Rural Sanitation*

YOUR committee has decided that the advance made in the field of rural sanitation through the utilization of relief labor during the last 2 years is sufficiently outstanding in importance to become the subject of this report of the committee. The chairman has been instructed by the committee to present a brief résumé of accomplishments.

Community sanitation projects were developed in 24 states during the Civil Works Program. In at least 9 of these states it is understood to have been the first intensive program ever undertaken for promotion of the construction of sanitary privies. The maximum number of laborers employed at one time during the C.W.A. program was 35,000. All but 2 of the 24 states continued the program and 8 additional states instituted community sanitation projects with E.R.A. labor. In each of the 8 additional states it is also understood that the inauguration of these projects constituted the first intensive sanitary privy program undertaken.

The popularity of the program is indicated by the fact that W.P.A. community sanitation projects calling for

* Report of the Joint Committee of the Conference of State Sanitary Engineers, the American Society of Agricultural Engineers and the American Public Health Association.

STATES ENGAGED IN THE PROSECUTION OF THE COMMUNITY SANITATION PROGRAM

24 Under C.W.A.	*30 Under E.R.A.*		*39 Under W.P.A.**	
Alabama	Alabama	Missouri	Alabama	Missouri
Arkansas	Arizona	Montana	Arizona	Montana
Delaware	Arkansas	North Carolina	Arkansas	New Mexico
Florida	California	New Mexico	California	North Carolina
Georgia	Florida	North Dakota	Colorado	North Dakota
Illinois	Georgia	Ohio	Delaware	Ohio
Indiana	Illinois	Oklahoma	Florida	Oklahoma
Kansas	Indiana	Oregon	Georgia	Oregon
Kentucky	Iowa	South Carolina	Idaho	Pennsylvania
Louisiana	Kansas	Tennessee	Illinois	South Carolina
Maryland	Kentucky	Texas	Indiana	South Dakota
Michigan	Louisiana	Utah	Iowa	Tennessee
Mississippi	Maryland	Virginia	Kansas	Texas
Missouri	Michigan	Washington	Kentucky	Utah
North Carolina	Mississippi	West Virginia	Louisiana	Virginia
Ohio			Maryland	Washington
Oklahoma			Massachusetts	West Virginia
Pennsylvania			Michigan	Wisconsin
South Carolina			Minnesota	Wyoming
Tennessee			Mississippi	
Texas				
Virginia				
Washington				
West Virginia				

* New Jersey has submitted projects to W.P.A. for approval.

approximately 60,000 laborers have been approved in 39 states.

During the C.W.A. program and through June 30, 1935, the installation of 504,212 sanitary privies has been reported by state health departments. In addition, the following items have been reported: (1) septic tanks, 5,442; (2) privies repaired, 44,680. It is estimated that there will be 3,000,000 sanitary privies constructed under the W.P.A. program.

It is exceedingly fortunate that the Committee on Environmental Sanitation provided standard plans and limiting specifications in its report presented to and adopted by this body in 1932. With the standard thus provided, uniformity in sanitary privy construction practice has been advanced to an extent and with a rapidity that could probably not have been accomplished in any other manner.

The education of the public in the importance of the sanitary disposal of excreta has been advanced many years in this short period. The interest of public health officials, and engineers in particular, has been greatly increased in rural sanitation. In all, approximately 3,000 persons have been engaged in the promotion and supervision of the program. Fully 500 of this number have been engineers. It is believed that the extent to which engineers have participated in the program accounts for the high quality of work accomplished and the advances that have been made in perfecting details of construction.

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American Public Health Association
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Shellfish*

LAST year we suggested that the Laboratory Section assist the Public Health Engineering Section in improving methods for the bacteriological examination of shellfish and that such methods include the examination of the entire shell contents. Some of the members of our committee and some of the members of the Laboratory Section committee have done some experimental work during the year employing a modified Eijkman method in an endeavor to find a method of examination which would report only the presence of those organisms in shellfish which were of true fecal origin.

The development of such a method, in our present state of knowledge, would help to remove suspicion from shellfish which under present methods of examination show evidence of high contamination, but which are so far removed from sources of fecal contamination that they could not possibly be affected thereby. The results of this study as carried out by members of our committee were somewhat disappointing. More detailed report on the results of this study will be found in the papers presented at the symposium on shellfish at the Milwaukee meeting of this Association this year.

The results of shellfish cleansing, particularly as applied to hard clams (*Venus mercenaria*) and soft clams (*mya arenarius*) continue to be satisfactory. More rapid progress could be made in this field—as pointed out last year—if a quick, simple, and positive method for disclosing the presence of pathogens in shellfish were available. One of the members of our committee has been engaged in making extended studies of shellfish and water scores aimed towards finding correlations with various factors that might affect the scores. The results of this study will probably be presented at a later time.

In some of the states increased activity in shellfish sanitation surveys and studies has been possible because relief funds were made available.

The further employment of funds for such purposes may make possible the collection of important data bearing upon the cleanliness of shellfish areas and the extent to which domestic and trade wastes may affect such areas.

Efforts to improve our present laboratory method of shellfish examination should be continued in the hope that a more precise and more useful method may be developed so as to make possible a more scientific sanitary control of the shellfish industry than is now possible.

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* Report of the Public Health Engineering Section Committee.

The Application of Limiting Standards of Pollution of Waterways*

THERE is an increasing trend toward the establishment of standards relating to practices and procedures in practically all branches of public health and sanitation. A survey of the activities of the various committees of the Public Health Engineering and other Sections of the American Public Health Association, particularly during the past two decades, clearly shows this tendency both as regards public health administration and methods of technical control. Accordingly, in the preparation of this report of your Committee on Waterways Pollution, it was decided that a summary of the general situation as regards the establishment and application of standards of purity or cleanliness for surface waters would be desirable, with the object of stimulating additional interest in and support for such developmental work in this field as is considered practical. To accomplish this purpose, a review of literature pertinent to the subject was undertaken, such data and information as are obtainable being supplemented by that from correspondence, unpublished reports, and memoranda which were kindly made available to the committee.

It hardly needs mentioning that views on standards applying to pollution were found to be quite varied. Undoubtedly there is much to be learned, and it is an open question whether regulation of waterways pollution in some of its many aspects can or need be placed on such a definite basis as may be interpreted from the term "standards." Yet in certain phases, notably as regards limits of pollution of sources of public water supplies, there appears to be both a reasonable basis and definite need for establishing standards. What is sought in this report is simply to set forth a general survey of the situation and make suggestions relative to future procedure in formulating and putting into effect adequate standards of cleanliness for our water courses.

Many of those called upon to administer anti-pollution laws and regulations are confronted with questions as to the requirements that must be met in safeguarding public interests in our waterways. This has directed attention toward establishment of limiting degrees of pollution or standards of cleanliness for lakes, streams and tidal waters. Attempts to develop standards for general application are known to date back at least to the activities of the Royal Commissions on Rivers Pollution in England, described in reports of the First Commission in 1865, and the Second Commission in 1868, along with reports of succeeding Commissions between 1870 and 1875. In the United States attention was first given to this phase of the general problem of waterways sanitation when in 1872 the Massachusetts Board of Health began a study of sewage disposal and stream pollution in that state, leading to the well known series of investigations at the Lawrence Experiment Station.

* Progress Report of the Committee on Waterways Pollution of the Public Health Engineering Section.

Since these pioneering efforts to arrive at an understanding of the technical aspects of water pollution and its control, much thought has been given by various authorities to this question of standards. The literature on sanitation includes numerous references covering standards that have been established or proposed for either local or general application in connection with waterways pollution. The underlying fault or shortcoming of a number of those adopted or advocated in the past has been the lack of a sufficiently comprehensive background of properly coordinated scientific information or facts to support the standards if the matter came to a test in the courts or otherwise. The situation is very well expressed in a report for 1934 [1] of the Committee on Research of the Federation of Sewage Works Associations, as follows:

> The late Professor William T. Sedgwick was fond of describing standards in sanitation as "devices to save lazy minds the trouble of thinking," which well describes the situation of his day, when a standard was generally no better than a pure guess by one worker easily seized upon, quoted and requoted by others until it assumed the semblance of authority. Something of this same method still remains in certain fields of modern sanitation, but with the development of standards committees in our great national organizations and the consequent critical discussion of current methods, together with the accumulation of scientific data upon which to base these discussions, and a formulation of a joint opinion, standards in the true sense of general agreement of opinion by expert and experienced technicians, are assuming an increasingly important rôle.

Broadly classified, the standards applying to waterways pollution set forth in the literature could be included under one or more of the following headings: those specifying—

1. Certain characteristics for sewage and other effluents that may be permitted to discharge into water courses
2. Dilutions necessary in receiving bodies of water
3. Limiting degrees of water degradation, governed by uses made of the waterways, and defined in units of readily determinable physical, chemical, biological, and bacteriological qualities of the waters

The earlier standards, such as set forth in the reports of the British Royal Commissions on Sewage Disposal and as formulated by Hering, Stearns, Hazen, Goodnough, and others, in specifying the number of cubic feet per second of stream flow required for the inoffensive disposal of sewage contributed per 1,000 population, can be grouped largely under the first two headings. It is evident that these standards were developed with the prevention of nuisances as the primary purpose, rather than the safeguarding of public water supplies.

Developments in recent years would indicate that the trend in standards of waterways pollution is toward the third classification, where all or major uses of the waters are considered. Based largely upon the comprehensive studies of the U. S. Public Health Service, there is now a tendency toward definitely limiting the *B. coli* content allowable in waters usable as sources of public water supply. For instance Jordan [2] states:

> The approach to a density limit of sewage organisms in a raw water is definite. Various expressions of this have been made, but none so definite as that of Streeter at the close of his long series of studies of performance of purification plants. Whether or not one agrees with the reasoning, he at least must defend against the indictment the use of any raw water whose average *B. coli* content exceeds 5,000 per 100 ml. It is reasonable to expect that in any future revision, the Treasury Standard limitations will not only be set as they are now upon the quality of finished waters, but also may be suggested as to limiting average *B. coli* index of raw waters.

Two types of standards (1 and 3 of the foregoing classification) are included in the interstate compact proposed by the Tri-State Treaty Com-

mission[3] of New York, New Jersey, and Connecticut for the abatement of pollution of the harbor and coastal waters of the Metropolitan Area of New York City. This included a recommendation that the waters be divided into two general classes, namely, Class A waters, which are used primarily for recreation, shellfish, and culture of fish life; and Class B waters, which are not expected to be used for such recreational and shellfish propagation purposes. The proposed compact also provided that all sewage tributary to Class A waters shall be subjected to a degree of treatment sufficient to remove all floating solids and at least 60 per cent of the suspended solids to effect a reduction of organisms of the *B. coli* group so that the probable number of such organisms shall not exceed 1 per c.c. in more than 50 per cent of the samples of effluent tested by the presumptive method, and to maintain in the general vicinity of the point of discharge at a depth of about 5 feet below the surface of the water a dissolved oxygen content of not less than 50 per cent saturation during any week of the year. Under Class B waters the specified treatment requirements for sewage were such as to secure removal of all floating solids, at least 10 per cent of the suspended solids, avoid formation of sludge banks, and effect a reduction in the oxygen demand of the sewage sufficient to maintain in the general vicinity of the point of discharge a dissolved oxygen content of not less than 30 per cent saturation during any week of the year.

An excellent review of the trends in water pollution standards has been prepared by Streeter,[4] in which he concisely summarizes the present situation as follows:

> The older standards, based on fixed dilution ratios, are being superseded by more definite specifications of certain indicators of relative cleanliness, such as bacterial content, dissolved oxygen, oxygen demand, and known toxic chemical substances. Where the public health is involved, limiting standards of bacterial quality, notably with respect to the density of organisms of the *B. coli* group, are becoming of primary importance, though in some instances limitation of chemically toxic substances is almost equally significant. At the present writing a reasonably firm empirical basis exists for the formulation of working standards of pollution for practically all waterways in this country used as sources of public water supply. This should be done in the near future, so as to coördinate efforts in the various states toward a rational and uniform policy in dealing with this problem, especially in its interstate aspects.
>
> A fairly good basis also now exists for the formulation of tentative standards of pollution for shellfish growing areas, and for natural bodies of water utilized for public bathing. In the latter case, however, caution will be necessary in attempting to meet a bathing water standard in some natural waterways subject to noncontrollable pollution by surface drainage water and storm sewage. In some of these instances, at least, the extent of dilution provided would not be sufficient to permit conforming to such a comparatively rigid standard, even if the effect of all the dry-weather sewage entering the waterway were to be removed through adequate treatment.
>
> Where the public health is not involved, the minimum dissolved oxygen standard appears to be the most simple and readily applied one for general application, though a distinct trend toward oxygen "balance" criteria is apparent. Some question remains as to just where the minimum oxygen level should be placed in specific cases, with a strong tendency to fix it at not lower than 3 or 4 p.p.m. and at even higher concentrations in some cases. As organic sludge deposits are particularly harmful to fish life and tend to exert a disproportionately heavy oxygen demand on overlying bodies of water, a definite need exists for standards of sewage and industrial wastes treatment adequately stringent to result in practically eliminating these deposits from all streams and lakes.

Through an inquiry directed to chief sanitary engineers of 25 state departments of health, Streeter found that beyond the U. S. Treasury Department Standard for drinking waters, there are no standards of cleanliness for waters devoted to various uses that

are generally accepted; that where other standards are applied, except as related to limiting degrees of pollution for sources of purified water supplies, these standards are not based on systematic observational data; and that there appears to be a fairly widespread sentiment favorable to the more general use of water quality standards, if they can be based upon such scientific data as to justify their acceptance with every confidence.

Considering the varied uses of waterways, such as for public water supply, watering of stock, industrial water supply, propagation of fish, shellfish, and other aquatic life, recreational purposes, including bathing and boating, navigation, and agricultural development involving irrigation, it is apparent that there is a large task to be performed before this condition for general acceptance of standards can be met. Harmful effects of pollution pertaining to these uses must be properly evaluated and limits defined in suitable physical, chemical, biological, and bacteriological units. The relation one to the other of the various units must be more thoroughly established. This is essential to intelligent application of any general standards to waterways having more than one major use. It is evident that only through the coöperation and careful coördination of results of research conducted by sanitary engineers, laboratory workers, epidemiologists, fish culturists, and many others will it be possible to proceed satisfactorily toward the establishment on a firm scientific foundation of acceptable standards of cleanliness for lakes, streams, and tidal waters.

To facilitate carrying out the necessary research and to provide ways and means for collecting, coördinating, and disseminating the results obtained, it is considered most desirable that some definite agency of the federal government be designated and provided with adequate funds to undertake the task. That the problem is of nation-wide importance is evidenced in many ways, and is recognized in Part III of the *Report of the National Resources Board to the President*,[5] which under the heading of water planning deals with the problems of waterways pollution. As the only comprehensive studies of a scientific nature on waterways pollution performed by the federal government have been conducted by the Office of Stream Pollution Investigations of the U. S. Public Health Service, and as it is believed to be well constituted to carry out the work effectively, your Committee on Waterways Pollution recommends that this Section go on record as urging favorable consideration by the appropriate authorities to the designation of this agency as a national clearing house for research and other activities necessary in the formulation and harmonizing of standards of water quality.

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DISCUSSION DEALING WITH LIMITING STANDARDS OF WATERWAYS POLLUTION

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THE subject of limiting standards of pollution for waterways devoted to various uses has been considered for many years, dating back to the work of the Massachusetts Board of Health in the 1880's and of the British Royal Commission on Sewage Disposal in the late 90's. With the recent increase in stream pollution throughout the United States and the growing public consciousness of the problem, interest in this subject has been revived on the part of those sanitary authorities who are faced with the possible institution of extensive stream cleaning programs in the near future. Consideration of this subject by a committee of this Association is, therefore, both timely and profitable.

In connection with a survey of national water resources made during the present year by the Water Resources Section of the National Resources Board, the present situation with respect to the use of water pollution standards in this country was reviewed for the board in a special report prepared by the speaker. A canvass was made of existing practice in the application of such standards in some 25 states, representing a fair cross-section of the country. The results of this canvass, together with a review of recent literature on the subject, have been taken as the basis of the present discussion.

In a general way, the results of the canvass indicated: (1) that aside from the Treasury Department drinking water standard, no standard of quality or cleanliness for waters devoted to various uses has any widespread degree of acceptance among the several states; (2) that other standards, where applied, are diverse in their requirements and with one exception are not based on extensive or systematic observational data; and (3) that with a few exceptions there appears to be a fairly widespread sentiment favoring the more general use of water quality standards, provided that they can be based on a sufficiently firm scientific foundation to justify their general acceptance.

WATER SUPPLY STANDARDS

From a public health viewpoint, the most important phase of the subject has to do with standards of quality for public water supplies and their sources. For domestic water supplies, the present Treasury Department standard has been a widely useful criterion, despite the fact that it was originally intended for application only to drinking and culinary waters supplied by the interstate carriers. With a few minor amendments, principally those designed to eliminate and restrict certain harmful chemical substances not included in the present requirements of the Standard, it can be readily extended as a suitable criterion of safety for all public water supplies. For industrial water supplies, requirements are so variable that no general standard of quality seems to be applicable. Progress in studies of this phase of the subject is being made by the U. S. Geological Survey under the direction of Chief Chemist W. D. Collins and by a subcommittee of the National Water Resources Committee headed by S. T.

Powell, who has made a particular study of boiler waters in this connection.

The matter of limiting standards of pollution for sources of purified domestic water supplies has been studied for a number of years by the U. S. Public Health Service, in connection with its stream pollution investigations. The results of this study, which involved extensive observational surveys of over 30 municipal filtration plants and the operation of a specially constructed experimental filtration plant for about 5 years, have led to the recommendation of a definite limiting standard of pollution for raw waters requiring filtration and for waters requiring only simple chlorination.[1]

For raw waters requiring filtration, the recommended standard would provide: (1) that the average *B. coli* index, when considered over a significantly long period of time, such as a year, should in no case exceed 5,000 per 100 c.c. and (2) that the *B. coli* index on individual days should not exceed 20,000 per 100 c.c. for more than 5 per cent of the same period, in which case the limiting average index might be as high as, or something less than 5,000 per 100 c.c., according to the character of the source and variability of water taken from that source. For waters requiring only chlorination, the average *B. coli* index should not exceed 50 per 100 c.c., or a figure sufficiently lower so that an index of 400 per 100 c.c. would not be exceeded for more than 5 per cent of the time. Both of these standards are based on the assumed production of purified effluents meeting both requirements of the Treasury Department *B. coli* standard. The term "index" may refer either to the Phelps number or to the "most probable number" of *B. coli*.

The standard recommended is now being followed with various modifications by several state health departments in fixing requirements for the quality of sources of purified municipal water supplies. Incidentally, it is the only limiting criterion of water quality thus far developed, which, as far as the speaker knows, is based on extensive and systematic observational data. For general use, it should be amended so as to provide for the restriction of certain harmful and taste producing substances which cannot be effectively removed by ordinary water purification processes.

Among other water quality standards having a more or less direct bearing on the public health are those which have to do with recreational uses of waterways, such as bathing and fishing, with certain agricultural uses, notably stock watering and irrigation of food crops intended for human consumption, and with the maintenance of general cleanliness and freedom from "nuisance" in streams, which is closely related to the suitability of bordering land areas for healthful dwelling places.

In the several fields of application of these standards, wide divergences in opinion and practice are found among the various states. This is particularly true of bathing water standards, which range from a limiting *B. coli* index as low as 50 per 100 c.c. to one as high as 1,000 per 100 c.c. Minimum dissolved oxygen requirements for the maintenance of aquatic life also show quite a diversity, varying from as low as 2.0 to 5.0 p.p.m. or more. Some fish experts, notably Dr. Hubbs of Michigan, hold that no definite dissolved oxygen minimum can be set, as the killing limit varies with temperature and various constituents of sewage have different effects on fish. Hubbs expresses a preference for an oxygen reserve standard, in which a fixed excess of dissolved oxygen over oxygen demand is specified, such as, for example, 3 p.p.m.

Standards of cleanliness for waters used in agriculture have been adopted in only a few of our states, notably in California, Colorado, Maryland, and the southwestern group. For irrigation waters applied to edible vegetable crops, the most definite standard is in Colorado, where such waters must not contain more than 1,000 coli-aerogenes bacteria per 100 c.c. Opinions vary widely as to the quality requirements of waters used for stock watering. At one end of the scale is the view that domestic cattle should be served only with water fit for human consumption. At the other end is the view that harmful effects of sewage on cattle are slight unless it contains chemical poisons or affects the protein intake of food. In Maryland, sewage entering streams used for stock watering must be chlorinated.

The history of efforts to set up standards of general cleanliness for polluted bodies of water extends back over a period of more than 25 years to the formulation of the British Royal Commission standard for sewage effluents and the various dilution standards recommended for use in the United States. Among the 25 states recently canvassed, only 4 have any definite standards in this respect, though several other local standards have been proposed in recent years. Among these is the standard suggested by Howson and Burdick for the Illinois River, in which the minimum dissolved oxygen content would be not less than 3 p.p.m. nor less than the 5 day biochemical oxygen demand at 20° C. This is similar in principle to the reserve oxygen, or "fish zero" standard proposed by Hubbs. Another recent standard set up for localized application is that of the Tri-State Pollution Commission for waters bordering the greater New York metropolitan area. This standard divides the waters into two classes, A and B, with considerably higher requirements for Class A than for Class B, the former being set aside for bathing and other recreational purposes. For Class A waters, among other requirements, the *B. coli* index is limited to 50 per 100 c.c., and the dissolved oxygen minimum to 50 per cent of saturation. For Class B, the oxygen minimum may be as low as 30 per cent of saturation.

Although it is possible that no fixed standards of the kind would be generally applicable to all classes of waters, there seem to be certain basic requirements that are common to all waters where freedom from "nuisance" conditions and their utilization for recreational purposes are desired. One requirement is the removal of all, or practically all, of the settleable organic solids from sewage and industrial wastes discharged into such waters, in order to prevent the formation of sludge deposits, which are detrimental to normal stream life. It is also agreed that for the prevention of "nuisance" or other undesirable conditions, the dissolved oxygen content should not fall below a certain minimum, and a favorable "oxygen balance" should be maintained. The precise location of these optima to meet the conditions of various uses of waterways still remain to be defined, in so far as their general acceptability is concerned.

In addition to these basic requirements, are others to be considered, notably with respect to the freedom of waters from biological growths resulting from the discharge of sewage and sewage effluents, the presence of certain toxic substances detrimental to maintaining a normal biological equilibrium in natural bodies of water and possible effects of oils and tarry substances on the natural life and purification capacity of these waters and their normal use by riparian dwellers.

The present status of water pollu-

tion standards and their application in this country may be summed up very briefly as follows: The use of such standards has been limited thus far to a comparatively few states and has been marked by a general lack of agreement as to their specific requirements for particular kinds of water utilization. As the result of the virtually unrestricted pollution of our waterway systems which has been permitted in the past, conditions in many of these waterways have become so serious that demands for their relief are becoming increasingly widespread and insistent. If these measures are to be both effective and economical, they should be planned and executed with a view to meeting definite limiting standards of pollution, fixed according to the requirements of each major water use involved and based on a scientific study of these requirements.

At the present writing, aside from the Treasury Department drinking water standard, which has become established as a reasonably safe criterion through long and wide experience, none of the standards mentioned in connection with this discussion has at present any widespread degree of acceptability or actual use. Of these other standards, only one, so far as is known to the speaker, has been recommended as the result of any systematic and comprehensive observational study. At the same time, virtually all of these standards are of such a nature that, once established on a scientific basis, they should be capable of fairly general applicability, because they have to do with water quality requirements which are common to all individuals and practically all situations of a given class. It is hardly conceivable, for example, that safety requirements for drinking or bathing waters would vary to any appreciable extent among several groups of individuals, wherever they might be located, or that the essential subsistence requirements for given species of fish or other aquatic life would vary widely in different bodies of water.

The present need, then, seems to be that some public health research agency experienced in water pollution problems and having at its command the necessary personnel and working funds, undertake a thorough study of the limiting requirements to be met for various kinds of water use, with a view to recommending standards based on such requirements. A study of this kind, if adequately planned and financed, would reveal more definitely the stronger and weaker points of standards now in use and would inevitably lead to their improvement and adaption for wider application. This general method of approach seems to be greatly needed at present in considering the question of water pollution standards, which too often in the past have depended on individual opinions backed by inadequate supporting data. If this Section of the American Public Health Association can lend its aid toward such an effort, it will be rendering a notable service to the cause of intelligent waterways pollution control in this country. The action of your committee in bringing the matter up for discussion at this time is most commendable.

REFERENCE

1. *J. Am. W. W. Assn.*, Sept., 1935, pp. 110–119.

To Study Nursing Service in State Departments of Health*

THE Committee to Study Nursing Services in State Departments of Health was set up as a joint committee because the work followed a study of State Nursing Services made by the National Organization for Public Health Nursing in 1931 and also because the question involved represented the interest of both groups. A large part of the statistical service has been given by Anna Miller, of the National Organization for Public Health Nursing staff, and without such valuable help the committee could not have functioned effectively.

The committee conceived its broad purpose to be the strengthening of nursing service in state departments of health. Its more immediate objectives were defined as three:

1. Collection of facts regarding the organization functions and practices in state nursing in the several states
2. Study of the collected data
3. Interpretation of the findings to determine whether the different situations offer comparable problems which may have some solution through the formulation of fundamental principles in the organizing and functioning of state nursing services

COLLECTION OF FACTS

Facts were collected through the medium of a questionnaire which included in its scope only such items as dealt with organization, functions, and methods. This limitation as to scope seemed desirable in order not to duplicate effort, because another joint committee to study personnel practices in states had its plans well under way. The two committees cleared plans and decided to exchange information. The questionnaire of this committee was sent to the 48 states and, in addition, to the District of Columbia and to Hawaii. All but three states, Delaware, Colorado, and South Carolina, returned the questionnaires.

The information received has been tabulated and obvious inconsistencies have been checked through correspondence. The data now available are sufficiently accurate and complete for the general purposes of the committee; but if the information were to be summarized by states following the form of the National Organization for Public Health Nursing study of 1931, the summary should very obviously be submitted to each state for final checking and approval. At the present time, the committee does not believe that such a specific summary is indicated, although a comprehensive summary of the answers to each item contained in the questionnaire might easily be prepared for distribution to the states which participated in the study. Several states indicated their interest in a complete report of work in other states so identifying information may have its value.

STUDY OF DATA

The information collected was carefully studied by the separate members of the committee and finally in meeting. It is considered significant that the interest of the committee increased during the 3 hour conference which was held just before the Annual Con-

* Report of the Joint Committee of the National Organization for Public Health Nursing and the American Public Health Association.

ference of the American Public Health Association in Milwaukee. The committee was unanimous in its belief that the study offered many possibilities which had not been thoroughly sounded. Sufficient refinement of the tabulation has not been made to include in this report a summary of the statistical information upon which the tentative conclusions of the committee are based. It is hoped that the following general statement will be accepted before the complete report is offered:

There are striking similarities in concept of function and in the concrete problems as outlined by the various states; on the other hand, there are wide divergencies in organization and in the methods by which similar problems are met. These factors offer great opportunity for further productive study. It is upon this tentative generalization based on the study of factual information that the committee offers its present conclusions:

1. The major problems seem to point to those of organization, budget, and personnel and are therefore administrative in character.
2. From the evidence at hand, there is a similarity in purpose in relation to public health nursing in several states to justify further study as to fundamentals in organization, functions, and methods.
3. The committee recommends the continuation of the study with the thought that the work ought to be continued in coöperation with the Nursing Committee of the Committee on Administrative Practice.

MARION SHEAHAN, *Chairman*
MARGARET EAST
OLIVIA PETERSON
PEARL MCIVER (*ex officio*)
ALMA HAUPT,
Representing N.O.P.H.N.

Accident Statistics

IN the absence of the Chairman, no formal report of the Committee on Accident Statistics was presented at the annual meeting in Milwaukee. The Chairman of the committee presented by letter a statement reviewing the past work of the committee, its inactivity during the past year, and a statement of possible problems that the committee should consider in the future.

It was suggested that the committee continue its previous promotion of the collection of supplemental data on fatal accidents by official departments of health and to continue its coöperation with non-official agencies such as the National Safety Council and the American Museum of Safety in the collection of accident statistics. The statement of the Chairman referred to the many agencies in the federal government that were collecting statistics on one or another kind of accidents but that no single federal agency is responsible for putting these data together. Dr. Louis I. Dublin recommended that the Vital Statistics Division of the Federal Bureau of the Census consider as one of its major responsibilities the publication of complete annual statistics of accidents. He referred to the excellent work of Dr. Earle G. Brown of Kansas in analyzing data available on fatal accidents in Kansas.

The Section referred the statement of the Chairman to the Section Council which decided to continue the committee appointing a new chairman to take the place of Dr. Fales who had requested to be relieved of the chairmanship because of lack of time to carry on the work of the committee.

W. THURBER FALES, *Chairman*
GEORGE H. VAN BUREN
J. V. DE PORTE
T. F. MURPHY
EARLE G. BROWN
I. C. PLUMMER
R. A. FORNEY, *Consultant*

Accuracy of Certified Causes of Death

THE report of the Committee on Accuracy of Certified Causes of Death and Its Relation to Mortality Statistics and to the International List was approved, in principle, by the Section on Vital Statistics of the American Public Health Association, at its meeting in Milwaukee, Wis., in October, 1935.

This report presents the committee's recommendations as to—(a) what titles of the present (Fourth) revision of the *International List of Causes of Death* should remain therein; (b) what terms should be listed under each title; (c) what terms should be transferred from one title to another. The full report was published in the *Public Health Reports*, September 13, 1935, and will appear as *Reprint No. 1706* from the *Public Health Reports*.

HAVEN EMERSON, *Chairman*

Stillbirths*

THE conclusions and recommendations of the Sub-committee on Stillbirths are offered for consideration of the Section on Vital Statistics for discussion before submission to the Committee on Research and Standards.

On approval by the latter standing committee of the Association, the Committee on Accuracy of Certified Causes of Death will represent the Association in conferences in this country and in Europe in preparation for the revision of the *International List of Causes of Death* which is planned for 1938 in Paris.

Before we can hope effectively to reduce the fetal and maternal losses, we must have adequate information with regard to the magnitude of the problem, the variations in prevalence, and the underlying fetal and maternal conditions associated with stillbirth mortality. The information is within the possibilities of statistical tabulation and analysis. Completeness of registration, comparability of original records, and uniformity in the use of terms and in registration practice are prerequisites.

Stillbirth registration is recognized as grossly incomplete in practically all sections of the country. Glaring lack of uniformity is found in the minimum period of uterogestation for which stillbirth registration is required. Variation also exists in the evidence of life used to distinguish between live and stillbirths; for although most states use the American Public Health Association rule No. 19 of Statistical Practice as amended in 1913, few have included it in recent instructions to certifying physicians. There is not in common use a list of causes, maternal and fetal, ante- and intra-partum, which lead to the occurrence of stillbirths.

To remedy these defects your committee recommends: Active stimulation of the registration of stillbirths, both as separate projects and in connection with campaigns for the improvement of registration of live births; the promotion of uniform state laws and regulations with regard to the minimum period of uterogestation to be used for recording, and the evidence of life to be used in distinguishing between live and stillbirths. Your committee also recommends the approval by the Section on Vital Statistics and by the Association, on recommendation of the Committee on Research and Standards, of a form of certificate of fetal death, and a list of terms to be used as a basis of a table of stillbirths by cause to be included in the annual report on Birth, Stillbirth, and Infant Mortality Statistics of the U. S. Bureau of the Census, and to be published by the states and by the larger cities in their annual reports. It also recommends that the *Physician's Pocket Reference* to the *International List of Causes of Death* issued by the Bureau of the Census be expanded to include a section on stillbirths which will emphasize the importance of stillbirth registration, give the definition of stillbirth, and the evidence of life to be used in distinguishing between live and stillbirths as recommended by the American Public Health Association in Rule 19, 1908,

* Report of the Sub-Committee on Stillbirths of the Committee on Accuracy of Certified Causes of Death. This report is presented for discussion prior to its adoption.

and the resolution of 1913, together with a list of fetal and maternal causes of stillbirth.

The following five matters were considered by the Sub-committee on Stillbirths to require clarification and formal agreement for purposes of public declaration of policy by the public health profession as represented in the American Public Health Association:

A. Definition of live and stillbirths
B. Elements of a uniform state law requiring the reporting of stillbirths
C. A standard certificate of stillbirth
D. A list of causes of stillbirth, whether determined in the fetus or related to conditions in the mother
E. Joint Cause Practice for the assignment of stillbirths

A.

Your committee wishes to call attention to the excellence in matter and form of the Report of a Committee on Definition of Stillbirth submitted to this Section in 1927 by Drs. Henry B. Hemenway, William H. Davis and Charles V. Chapin, which refers to correspondence in the Census Bureau by Dr. Cressy L. Wilbur in 1907, and with approval to No. 19 of Rules of Statistical Practice adopted by the Section on Vital Statistics of the A.P.H.A. in 1908, which reads as follows:

No child that shows any evidence of life after birth should be registered as a stillbirth.

In 1913 the following resolution was adopted by the Section on Vital Statistics:

Resolved, that the present Rules of Statistical Practice relating to stillbirths and premature births as adopted by the American Public Health Association in 1908, should be strictly followed by American registration offices, it being understood that the words *any evidence of life* in Rule No. 19 should include action of heart, breathing, movement of voluntary muscle.

It was further noted in this report of 1927 that,

So far as is known to the members of this committee, American statistical offices have been strictly observing this rule as amended by the resolution, except that the Province of Quebec has determined the fact of life after birth solely by the act of breathing.

The report of 1927 (Hemenway, Davis, and Chapin) considers in detail a communication from the Health Section of the League of Nations dated April 1, 1925, and refers with approval to No. 16 of the Rules of Statistical Practice (1908) which reads as follows:

Statement of viability or non-viability of an infant prematurely born shall not be considered in classifications.

The report further correctly states with admirable brevity that

The term stillbirth or deadbirth has universally been recognized as meaning dead at the moment of birth. Death means absence of life. As generally recognized the status of fetus terminates at birth, provided that the organism is alive at time of birth. Life of an animal is shown by heart beat, respiration or movement of voluntary muscle. This is general, and it applies to adults as well as to newborn children.

The report concludes with the recommendation that the existing rules of statistical practice regarding stillbirths be reaffirmed.

Rule No. 4 (4, 1908)—Stillbirths should not be included in deaths.

Rules No. 5 (5, 1908)—Children born alive and *living for any time whatever*, no matter how brief, after birth, should not be classed as stillbirths, even though reported by the attending physicians or midwives as "stillborn."

Rule No. 6 (6, 1908)—Wherever age, in days, hours or minutes, is reported for a "stillborn" child, or indicated by a difference between dates of birth and death, the registrar should secure a statement that will enable the case to be classified with certainty either as a stillbirth or as a death. If no additional information can be obtained, the statement of age should govern, and the case be compiled as a death, not as a stillbirth.

Rule No. 17 (17, 1908)—For registration purposes, stillbirths should include all children born who do not live any time whatever, no matter how brief, after birth.

C.

STANDARD CERTIFICATE OF STILLBIRTH
(See instructions on reverse side)

State File No._____
Registered No._____

1. PLACE OF BIRTH
County_____ State_____
Township_____ or Village_____
City_____ No._____ St._____ Ward
(If birth occurred in a hospital or institution, give its NAME instead of street and number)

How long has mother lived in this city or town, immediately prior to this birth_____yrs._____mos._____days. How long in U. S., if of foreign birth_____yrs._____mos._____days

2. Full name of child_____

3. Sex	If plural (4, Twin, triplet, or other_____ births (5. Number, in order of birth_____	6. Premature_____ Full term_____	7. Legitimate_____	8. Date of birth_____, 19__ (Month, day, year)

FATHER	MOTHER
9. Full name_____	18. Full maiden name_____
10. Residence (usual place of abode) (If nonresident, give place and State)_____	19. Residence (usual place of abode) (If nonresident, give place and State)_____
11. Color or race_____ 12. Age at last birthday_____(years)	20. Color or race_____ 21. Age at last birthday_____(years)
13. Birthplace (city or place) (State or country)_____	22. Birthplace (city or place) (State or country)_____
OCCUPATION: 14. Trade, profession, or particular kind of work done, as spinner, sawyer, bookkeeper, etc._____ 15. Industry or business in which work was done, as silk mill, sawmill, bank, etc._____ 16. Date (month and year) last engaged in this work_____, 19__ 17. Total time (years) spent in this work_____	OCCUPATION: 23. Trade, profession, or particular kind of work done, as housekeeper, typist, nurse, clerk, etc._____ 24. Industry or business in which work was done, as own home, lawyer's office, silk mill, etc._____ 25. Date (month and year) last engaged in this work_____, 19__ 26. Total time (years) spent in this work_____

27. Number of children of this mother (at time of this birth and including this child)_____ (a) Born alive and now living_____ (b) Born alive but now dead_____ (c) Stillborn_____

MEDICAL CERTIFICATE - CONFIDENTIAL

28. Period of gestation_____(weeks) 29. Was labor induced mechanically_____ Total duration of labor_____(hours)
(Include rupture of membranes, bag, bougie, and packings)

30. Was there an operation for delivery_____ Name of operation_____ Did operation precede fetal death_____
(Include low, mid, and high forceps; version and extraction; breech delivery; Cesarean; and craniotomy and other mutilating operations)

31. State the drugs and anesthetics given during labor and delivery_____

32. Did fetal death occur before labor_____ During labor_____ 33. Was the fetus macerated_____

34. Cause of stillbirth (a) Fetal_____
(See reverse side) (b) Maternal_____

CERTIFICATE OF ATTENDING PHYSICIAN OR MIDWIFE

I hereby certify that I attended the birth of this child, who was stillborn at_____P. M. on the date above stated.

(When there was no attending physician) (or midwife, then the father, householder, etc., should make this return)

(Signed)_____, M.D.
or_____Midwife
Address_____

Filed_____ 19__ _____
Registrar

Registrar

D.

UNITED STATES STANDARD CERTIFICATE OF STILLBIRTH

Definition of a stillbirth.—A stillborn child is one which shows no evidence of life after complete birth (no breathing, no action of heart, no movement of voluntary muscle). Birth is considered complete when the child is altogether (head, trunk, and extremities) outside the body of the mother even if the cord is uncut and the placenta still attached.

Why stillbirths should be registered.—Registration of all stillbirths is important: FIRST, on account of the great loss of human life occurring in the prenatal and natal periods; SECOND, on account of the relationship between fetal deaths and maternal mortality and morbidity. Although it is often impossible to determine the exact cause of fetal death, information regarding the associated conditions will lead the way to lowering the high fetal and maternal mortality. Important causes and associated conditions frequently found are shown below.

Instructions re Inq. 39. Cause of stillbirth (a) Fetal (b) Maternal.—Describe fully the conditions in the fetus and mother which were associated with the fetal death—i.e., conditions found in the fetus at the time of birth and diseases and conditions of the mother during pregnancy and labor. Indicate the condition you consider the primary cause of the fetal death. For example: The dead fetus was hydrocephalic; the mother had had a mild case of toxemia during pregnancy; the fetus would probably have been live born except for the hydrocephalus. Enter under Inq. 39: (a) Fetal—"Hydrocephalus—primary." (b) Maternal—"Toxemia."

CAUSES OF STILLBIRTH

A. CAUSES DETERMINED IN THE FETUS

1. Infection
   a. Syphilis
   b. Sepsis
   c. Other infectious
2. Asphyxia
   a. Separation of placenta
   b. Abnormalities of cord
   c. Other causes of asphyxia
3. Congenital malformations
   a. Hydrocephalus
   b. Anencephalus
   c. Spina bifida and meningocele
   d. Malformations of heart
   e. Other
4. Birth injury
   a. Malpresentations
   b. Difficult labor
   c. Other causes of physical injury of the fetus
5. Other diseases or conditions affecting the fetus primarily
6. Cause unknown

B. CAUSES AND CONDITIONS IN THE MOTHER ASSOCIATED WITH THE FETAL DEATH

1. Infectious and parasitic diseases
   a. Influenza
   b. Tuberculosis
   c. Syphilis
   d. Other
2. Cancers and other tumors
   a. Malignant and other tumors of the female genital organs
   b. Malignant and other tumors of other organs
3. Rheumatic, nutritional, endocrine, and other general diseases
   a. Acute rheumatic fever
   b. Diabetes
   c. Diseases of the thyroid and parathyroid glands
   d. Other
4. Diseases of the blood and blood-making organs
   a. Pernicious anemia
   b. Other
5. Chronic poisonings and intoxications
   a. Alcoholism
   b. Lead poisonings
   c. Other
6. Diseases of the nervous system and organs of special sense
   a. Psychosis
   b. Epilepsy
   c. Other
7. Diseases of the circulatory system
   a. Diseases of heart
   b. Diseases of arteries
8. Diseases of the respiratory system
   a. Bronchitis
   b. Pneumonia
   c. Other
9. Diseases of the digestive system
   a. Diseases of liver—specify
   b. Appendicitis
   c. Hernia, intestinal obstruction
   d. Other
10. Diseases of the genitourinary system—known to have preceded pregnancy
    a. Nephritis
    b. Other
11. Diseases of pregnancy and childbirth
    a. Sepsis—observed before delivery
    b. Albuminuria, eclampsia, and pyelitis
    c. Other toxemias—specify with or without convulsions
    d. Hemorrhages, ante- and intrapartum, due to placental abnormalities and other conditions
    e. Ectopic gestation
    f. Other accidents of pregnancy and childbirth—specify
12. Operations other than for delivery—specify
13. Conditions of the house interfering with normal labor
14. External causes
    a. Traumatism—blow, fall, shock, etc.
    b. Overwork
    c. Other—specify
15. Other diseases and conditions of the mother—specify
16. Cause unknown

Rule No. 18 (18, 1908)—Birth (completion of birth) is the instant of complete separation of the entire body (not body in the restricted sense of trunk but the entire organism, including head, trunk, and limbs) of the child from the body of the mother. The umbilical cord need not be cut or the placenta detached in order to constitute complete birth for registration purposes. A child dead or dying a moment, no matter how brief, *after* birth, was a living child, and should not be registered as a stillbirth.

Rule No. 19 (19, 1908)—No child that shows any evidence of life after birth should be registered as a stillbirth.

Rule No. 20 (20, 1908)—Stillbirths should not be included in tables of births or in tables of deaths. They should be given in separate tables of stillbirths.

Rule No. 21 (21, 1908)—It is not desirable that midwives be permitted to sign certificates of stillbirths.

Rule No. 22 (22, 1908)—Total births should include children born alive only, and headings of tables should state that stillbirths are excluded.

Rule No. 23 (23, 1908)—Whenever, under foregoing rules, a death should be registered, there should be a corresponding registration at some previous time of a birth; and whenever a stillbirth is registered it should be rigorously excluded from both the statistics of births and deaths.

The sub-committee at present reporting endorses each of the above rules of statistical practice as adopted by the Section on Vital Statistics in 1908.

While it is rare for a product of pregnancy of less than 28 weeks gestation to present evidence of life after complete birth (*i.e.*, action of heart, breathing, movement of voluntary muscle), such instances seem to be of undoubted authenticity and when this does occur it is worthy of special medical record and supporting comment. Under Rule 19, 1908, as amended in 1913, such products are, of course, registered as live births. The committee recommends that the information with regard to period of gestation be noted on the live birth certificate and the supporting comment be made a matter of special confidential record for medical purposes.

Unless gestation has advanced to the 5th month, *i.e.*, after 120 days, the product of pregnancy need not be reported as a stillbirth.

The term *abortion* should be reserved for the expulsion or extraction of the product of conception previous to its presumed viability, usually understood to apply to a fetus of less than 28 weeks from the assumed date of conception (6½ calendar, or 7 lunar months, at which time, in the races prevailing in the United States, the fetus will weigh 1,500 grams or 3¼ pounds and have a length of 35 centimeters or 14 inches).

The term *early abortion* should be applied only to expulsion or extraction of the product of conception prior to the end of the first 12 weeks of pregnancy.

The term *late abortion* should be applied to expulsion or extraction of the product of conception from the end of the twelfth week, or after development of the placenta and up to the 28th week of pregnancy.

The term *premature labor* should apply to the delivery of a fetus capable of surviving, and as usually understood, but not absolutely limited in fact, to apply to the product of conception after 28 weeks or 6½ calendar months or seven lunar months, which in American experience will usually have a weight of 1,500 grams or 3¼ pounds and a length of 35 centimeters or 14 inches.

B.

While the value to science and to society of an exact knowledge of all pregnancies, their duration prior to interruption, at any time prior to term can be logically maintained, nevertheless we believe practical considerations and the weight of administrative procedures and professional traditions are of such strength as to justify us in planning for and promoting registration

of only such stillbirths as are estimated to represent products of conception of 20 weeks' duration or more. This is the period recommended in the model birth registration law.

It is recommended that the influence of the American Public Health Association be exerted in all proper ways to bring concerted action of appropriate professional bodies and other organizations, so that state legislatures may be persuaded to enact new laws or amend existing statutes to require the registration of stillbirths, uniformly defined and by the use of a form approved by the Bureau of the Census for this purpose, and by the use of an approved list of terms describing the cause of the stillbirth comparable as far as practicable with the *International List of Causes of Death*, and that to this end the following organizations be approached for coöperative action in the matter—

U. S. Bureau of the Census
U. S. Public Health Service
Children's Bureau
State and Territorial Health Officers
Conference of State and Provincial Health Authorities of North America
American Medical Association (Sections on Obstetrics, Gynecology, Pediatrics, Preventive and Industrial Medicine, and Public Health)
American Academy of Pediatrics
Association of American Obstetricians and Gynecologists
Association of State Registrars
National Conference on Commissioners on Uniform State Laws
Life Insurance Companies

C. and D.

The text of the face of a suggested standard stillbirth certificate (C), with a list of approved causes of stillbirth printed on the back or reverse (D), is recommended.

E.

Your committee is eager for suggestions regarding this proposed certificate from all the members of the American Public Health Association who are interested in the registration of stillbirths. We believe, in many instances, two or more causes will be simultaneously reported on these certificates and that a manual of joint causes for stillbirths will be needed. No recommendations of a more exact nature are offered on this subject at present except that the experience of selected lying-in hospitals and a few cities be studied systematically to determine the general usefulness of the certificate, the types of causes which are reported, the extent of joint cause reporting, and the most practical means of encouraging uniformity in the practice of registration officials. Following the special studies mentioned above it will be feasible to prepare detailed instructions for certifying causes of stillbirth, and a list of undesirable terms. For the present the committee recommends that the instructions be limited to those shown on the back of the present form as follows:

Describe fully the conditions in the fetus and mother which were associated with the fetal death, *i.e.*, conditions found in the fetus at the time of birth and diseases and conditions of the mother during pregnancy and labor. Indicate the condition you consider the primary cause of the fetal death. For example: the dead fetus was hydrocephalic; the mother had had a mild case of toxemia during pregnancy; the fetus would probably have been live born except for hydrocephalus. Enter under item 34: (a) Fetal—Hydrocephalus—primary. (b) Maternal—Toxemia.

HAVEN EMERSON, *Chairman*
F. L. ADAIR
ETHEL C. DUNHAM
D. A. D'ESOPO
ELIZABETH C. TANDY

Registration of Births Out of Wedlock

THE Committee on the Registration of Births Out of Wedlock was appointed by the Council of the Vital Statistics Section in June, 1935. The problem entrusted to it presents two main phases: (1) the registration of children whose parents marry after the birth of the child, and (2) the registration of children whose parents do not marry each other.

The standard form of birth certificate calls for a statement regarding the legitimacy of the birth. If a child is born out of wedlock the question relating to legitimacy is answered "no." According to the laws of most of the states a child born out of wedlock becomes legitimate if his parents subsequently marry each other. When evidence to this effect is submitted to the registration office, the usual procedure consists of crossing out "no" and inserting "yes." The certificate, however, still bears on its face the fact that the child, now legitimate, was born out of wedlock. Several states provide that in such cases new certificates may be filed in which the birth is entered as that of a legitimate child. The committee questions the advisability of such a procedure since, from a strictly legal point of view, a certificate must show the facts as they are at the time of birth.

The committee recommends that the item relating to legitimacy be eliminated from the standard form of birth certificate since only in this way could the stigma of illegitimacy be removed from the child born out of wedlock who is later legitimatized through the marriage of his parents. It may be added that no reference to legitimacy is contained in the birth certificates used by New York City and the states of California, Massachusetts, and New Hampshire.

The committee is not yet in position to make any definite statements regarding the wider aspects of the problem. According to the practice prevalent in this country, the name of the father of an illegitimate child may not be entered on the certificate without his consent. Therefore, a certificate in which the father's name is not given indicates that the child is illegitimate. It is of interest to note that at least in two states (Minnesota and Wisconsin), bureaus of the state government take measures to ascertain the paternity of children born out of wedlock, and, when it is juridically established, the name of the father is entered on the certificate even though the mother remains unmarried.

In its future activities the committee will be guided by the concept which inspired the work of an earlier committee of the Section *—that no child born in this country should be handicapped because of the circumstances of his birth. This is the forthright position taken by the State of Arizona, whose statutes contain the clear-cut statement that "every child is the legitimate child of its natural parents." †

In the course of the coming year, the committee will endeavor to formulate definite recommendations to be presented at the next Annual Meeting of the Association.

* See Report of Committee on Registration of Births of Illegitimate and Adopted Children. American Public Health Association *Year Book, 1933–1934*, pp. 84–87.

† *Sec. 273. All children declared legitimate.* Every

child is the legitimate child of its natural parents and is entitled to support and education as if born in lawful wedlock, except the right to dwelling or a residence with the family of its father, if such father be married. It shall inherit from its natural parents and from their kindred heir, lineal and collateral, in the same manner as children born in lawful wedlock. This section shall apply to cases where the natural father of any such child is married to one other than the mother of said child, as well as where he is single. (The Revised Code of Arizona, 1928. Chapter 9, Bastards.)

J. V. DEPORTE, *Chairman*
W. THURBER FALES
SYBIL FOSTER
L. W. HUTCHCROFT

Resolution Adopted by the Vital Statistics Section

(Milwaukee, October 8, 1935)

RESOLVED: That the Section go on record in favor of amending the Model Law for Birth Reporting, so as to provide for omission from the original birth record the statement as to legitimacy of the child.

Confidential Inquiry Into Cause of Death

TWO years ago, a report was presented at a meeting of this Section on an experiment made in the Westchester County Health District to determine, first, the inaccuracy present under the existing system of death registration in the statement of two causes of death (namely, syphilis and alcoholism), and second, the reaction of the general medical profession to the use of a confidential form of death certificate. This experiment consisted of interviewing personally a group of 350 physicians, each of whom reviewed all of the death certificates he had signed during a period of 2 years and stated confidentially in which cases alcoholism or syphilis had been a leading or contributory cause of death. The investigator, a physician from the health department, also discussed with each doctor in the group the whole question of whether or not a confidential form of death certificate would result in greater accuracy than can be attained under the existing system. The results may be summarized as follows:

1. Additional data secured confidentially by personal visit for 5,299 deaths resulted in a death rate from syphilis approximately double the original recorded mortality from this cause and a corrected figure for alcoholism 57 per cent higher than the recorded rate. Syphilis was stated on the original certificate in approximately 49 per cent of the cases in which it should have been so certified, and alcoholism in 33 per cent.

2. Practically all of the 365 physicians reporting deaths in the Westchester County Health District during a 2 year period felt that confidential reporting would result in far greater accuracy in the statement of causes of death than does the present official system.

In view of these results and the apparent enthusiasm of the group of physicians concerned, it was decided to follow this experiment immediately with another. This second had as its major object to set up, if possible, a system of voluntary confidential reporting of causes of death. To this end a form was designed which allowed space for a confidential statement of the primary and contributory causes of death. A detachable part of the form which was finally accepted by the County Medical Society contained identifying information, specifically the date and place of death and the cause of death stated on the original certificate. In no place was the name of either the patient or the physician included. Although date and place of death was sufficient information to locate the official death certificate on record in the county office for comparison, the fact that the County Medical Society earlier rejected a form containing on the detachable section the name of the decedent in addition to the place and date of death would make it appear that physicians believed that they were not identifying their patients. Every effort was made to make it clear that the confidential statement could and would be checked with death certificates but that once this was done the identifying data would be detached from the rest of the blank and destroyed.

A number of blank forms and stamped return envelopes were supplied to every physician in the district with the request that one be made out and mailed to the County Commissioner

CONFIDENTIAL STATEMENT

PLACE OF DEATH .....

DATE OF DEATH .....

CERTIFIED CAUSE OF DEATH .....

.....

NOTE: This section is to be used only for determining that a confidential report is filed for every death. It will be detached immediately from the lower section and destroyed.

CAUSE OF DEATH (Confidential Statement)

CONTRIBUTORY CAUSES (Confidential Statement)

AUTOPSY FINDINGS—If Any (Confidential)

NOTE: This section is strictly confidential and will be used only for statistical purposes, and promptly destroyed.

Mail to the Westchester County Commissioner of Health, 148 Martine Avenue, White Plains, as soon as possible after the standard certificate of death is filed with the Registrar.

of Health for every death certificate signed, regardless of whether or not there was any additional confidential information to be given. The project thus roughly described was endorsed by the County Medical Society both through letters to each individual physician and through a notice published in the society's monthly bulletin.

The results were frankly a failure. For the first 2 months after the forms were sent out approximately one-third of the recorded deaths were also reported on confidential blanks. After that only a group of a dozen physicians continued to make reports, and the number of their deaths was negligible. Obviously to fill out and mail routinely a voluntary report of this sort, whose only use is statistical and theoretical, is more than can be expected of busy practising physicians, however interested they may be in possible results.

A total of 210 confidential blanks was returned before it became evident that the project in that form should be abandoned. Although this number is extremely small and could not possibly give results from which any general conclusions may be drawn, they have been analyzed with a view to shedding some light on the very significant question as to how much error, which can be discovered through a confidential inquiry of some sort, there is at present in recording causes of death. This small group of reports is probably fairly representative as far as it goes, inasmuch as it comprises reports on all of the deaths attended by a few physicians over a period of 2 or more months. To be sure, the physicians who reported were those who were especially interested in the project, but comparison with the original survey shows that they were not physicians who had under-reported syphilis and alcoholism any more than others. In a number of cases none of the deaths in a physician's practice were recorded with an incomplete cause on the official death certificate.

Analysis of the 210 confidential blanks reveals the following facts:

1. Information not included on the death certificate was stated on 62 or approximately 30 per cent of the returned confidential reports. In 14, or 6.7 per cent of the total number, this additional information would, if stated on the death certificate in addition to the information already there, result in a change of *International List* classification according to the *Manual of Joint Causes of Death*. Within this group of 210 deaths the following changes in assignment of cause of death resulted:

Syphilis deaths increased in number from 3 recorded deaths to 6. (This result, although based on small figures, checked closely with the results of the original survey.)

Cancer deaths increased from 25 to 28 (12 per cent).

Cerebral hemorrhage declined from 17 deaths to 14.

Deaths from diseases of the heart declined from 66 to 62.

2. Results which are of general interest but resulted in no change in classification were as follows:

Alcoholism was omitted as a contributory cause from the original death certificate in 7 cases. On no original certificate was it given as either a primary or contributory cause.

Syphilis was omitted from a total of 5 certificates. In 2 of these cancer and automobile accident were the primary causes and no change in classification resulted.

Drug addiction was included as confidential information in 2 cases.

CONCLUSIONS

The committee has set itself the problem of determining two facts, the first of which is the relative value of various methods of confidential inquiry into the cause of death. The projects in the Westchester County Health District have shown that through a direct personal contact with the physician information may be obtained successfully, but that a voluntary supplemental report, entirely separate from the death certificate and mailed to a different official agency, is not successful. The first is the most expensive and troublesome method of getting information, and the second the cheapest and easiest. It is hoped that within the coming year a modification and combination of the two methods may be attempted. For example, it might be feasible to mail to each physician at intervals of 3 months a list of the death certificates which he has signed during the period with a request that he state confidentially in each case the primary and contributory cause of death and return the list to the County Department of Health with the names detached. Some system of numbering such lists would have to be evolved inasmuch as it is essential in tabulating the statistical results to know exactly what group of original deaths has been included.

Once a suitable and reliable method of getting the information has been found the second problem is solved—namely, to determine what effect confidential and more complete information has on recorded death rates. At present the only fact that has been discovered is that in the Westchester County Health District the true death rate from syphilis is at least double the recorded rate. It remains to be proved what correction factor should be applied to this death rate in other communities or to the rates from other causes in Westchester and in other areas.

GAIUS E. HARMON, *Chairman*
MARJORIE T. BELLOWS, *Secretary*
MATTHIAS NICOLL, JR.
HAVEN EMERSON
COUNCIL OF THE VITAL STATISTICS SECTION

Forms and Methods of Statistical Practice*

THE report of the committee covered three subjects. The first discussed classification of sub-types of disease and included the following recommendation:

> That whenever a case or death is reported as due to smallpox, an effort be made to obtain from the diagnostician or epidemiologist a statement whether the disease was of the classical malignant type or of the benign type, and to indicate malignant cases or deaths in tables through an asterisk or other symbol leading to an explanatory footnote.

The second part of the report dwelt upon the desirability of promoting the use of selected age groups for expressing death and morbidity rates from certain diseases, *e.g.*, the earlier years of life for the communicable diseases of childhood. It was reported that work along these lines had been begun and that more specific recommendations would be presented at the next annual meeting. The use of standardized rates will also be considered.

The third part of the report consisted of provisionally recommended tables for monthly reports of State Health Departments. The subjects included natality, morbidity, and mortality. This report was presented by Dr. John Collinson as Chairman of the sub-committee on Standard Tables. Suggested table forms were given out at the meeting and discussed. A definitive report will be presented at the next annual meeting.

A. W. HEDRICH, *Chairman*
JOHN COLLINSON,
Chairman, Sub-committee
S. D. COLLINS
T. F. MURPHY
ELIZABETH PARKHURST
L. W. HUTCHCROFT.

* Abstract of Progress Report.

Residence Correction

THE Committee on Residence Correction (originally designated as the Committee on the Proper Allocation of Records) was appointed in October, 1926, at the Buffalo meeting of the American Public Health Association. In the course of the following nine years the need of allocating records of births and deaths according to residence (with certain exceptions in regard to deaths from tuberculosis, other chronic diseases, and external causes) has become generally recognized and has been adopted as a routine procedure by many states.

In 1934 the U. S. Bureau of the Census decided that beginning with 1935 its annual reports should be based on the resident and not, as hitherto, recorded numbers of births and deaths. The aim of the committee, namely, to bring about the establishment of a nation-wide system of residence correction having been accomplished, the committee asks that it be dissolved.

J. V. DEPORTE, *Chairman*
W. J. V. DEACON
W. THURBER FALES
A. W. HEDRICH
T. F. MURPHY
I. C. PLUMMER
G. H. VAN BUREN

Universities and Colleges in the United States and Canada Conferring Public Health Degrees

	Degrees Conferred
University of California	A.B. M.A. Ph.D. in Hygiene Dr.P.H.
Columbia University	M.S.P.H. Ph.D. in Sociology (Vital Statistics), Epidemiology, Sanitary Science, Bacteriology (Public health laboratory work)
Harvard School of Public Health	C.P.H.* M.P.H. Dr.P.H. Ph.D. in Hygiene
Johns Hopkins School of Hygiene and Public Health	C.P.H. Dr.P.H. Sc.D. in Hygiene Sc.M. in Hygiene
Massachusetts Institute of Technology	Ph.D. Dr.P.H. M.S. S.B. in Public Health S. B. in Public Health Engineering S.B. in Sanitary Engineering (The Department also awards the Certificate in Public Health)
University of Michigan	D.P.H. M.S.P.H.
University of Toronto, School of Hygiene	Ph.D. D.P.H.
University of Western Ontario	D.P.H.
Yale University, School of Medicine	M.P.H., Dr.P.H. } Offered by the Medical School M.S., Ph.D. } Offered by the Graduate School
Wayne University College of Medicine and Surgery	Dr.P.H.

* Harvard does not consider the Certificate of Public Health a degree.

Entrance Requirements for Graduate Degrees

University of California

Dr.P.H.—Graduate of an approved medical school.

Ph.D. in Hygiene—Candidates must have completed courses equivalent to those constituting the undergraduate curriculum in public health.

Columbia University

M.S. in P.H.—Graduate of a class A medical school with a degree of Doctor of Medicine or a graduate in arts or sciences having an acceptable degree of Bachelor of Science or Bachelor of Arts, and presenting evidence of having satisfactorily completed college courses in chemistry, physics, bacteriology or general biology, and mathematics. In addition such candidates should have completed a course in medical bacteriology similar to that given in the first year of the School of Medicine.

M.S. in P.H. majoring in Sanitary Science—Graduate of an approved engineering school, having a degree of B.S. in civil engineering, or its equivalent.

Harvard School of Public Health

C.P.H.—Evidence of having had satisfactory training in modern languages, inorganic, organic and biochemistry, biology, physiology, anatomy, histology, pathology and bacteriology. As a rule these requirements will be met by students possessing a bachelor's degree plus the first 2 years in an approved medical school.

M.P.H.—Satisfactory evidence of having received the M.D. degree, or its equivalent, from an approved medical school.

Dr.P.H.—Satisfactory evidence of having received the M.D. degree, or its equivalent, from an approved medical school. To qualify as a candidate, the student is required to pass an examination of the same type as that required to obtain the degree of M.P.H. It is intended to provide the assurance that all men receiving the Dr.P.H. are grounded in the four fundamental subjects and in the field most closely allied to their special interests.

Ph.D. in Hygiene—Properly qualified students in public health, who have no medical degree but who wish to secure a higher degree, may obtain a Doctor of Philosophy in Hygiene. This degree is granted by the university to men, or through Radcliffe College to women.

Johns Hopkins School of Hygiene and Public Health

C.P.H.—Graduate of approved medical school or graduate in arts or science, with adequate courses in physics, chemistry, and biology, and in the basic medical sciences, anatomy, histology, physiology and pathology.

Dr.P.H.—Applicant must be recommended by the head of the department in which he proposes to do his major work as an acceptable candidate. Students not personally known will be accepted only provisionally, final acceptance being conditioned upon the character of the work done.

In addition applicant must be:

a. Graduate of an approved medical school, who has had a liberal education as evidenced by a degree in the arts or the sciences or its equivalent, and who has completed the course leading to a C.P.H. or its equivalent.

b. Student who has fulfilled the requirements as to a liberal education and who has satisfactorily completed 3 years of this course in an approved medical school may be admitted to advanced standing which will permit him to complete the requirements for the degree in 1 year after graduation in medicine, provided arrangements are made to include in the medical course a sufficient number of courses which may be credited toward a C.P.H.; provided, also, that he is prepared, on admission, to enter upon advanced work in a selected major subject. During the year of residence, such a student, in addition to work in the major subjects, will be expected to take such of the courses leading to a C.P.H. as have not been absolved.

Sc.M. in Hygiene—*A degree in arts or science, or in medicine, from an approved school.

Certificates of the completion of adequate courses in physics, chemistry and biology.

Sc.D. in Hygiene—

a. *A degree in arts or science, or in medicine, with a reading knowledge of French and German.

b. Certificates of the satisfactory completion of adequate courses in physics, chemistry, and biology.

c. Certificates of the satisfactory completion of adequate courses in anatomy including histology, physiology, and pathology.

d. In exceptional cases, on the recommendation of the head of a department, a candidate may be accepted who offers in place of (c) satisfactory evidence of special training of an advanced character in subjects fundamental to his main field of work.

e. Not less than 1 year before being presented for the degree, the candidate will be required to pass an oral examination before a committee of the faculty. Final acceptance as a candidate for the degree will be conditioned upon favorable recommendation by the committee.

Massachusetts Institute of Technology

C.P.H.—Graduate in arts or science from a recognized institution or a medical degree from a class A medical school or have had professional training equivalent thereto.

Dr.P.H.—Graduate in arts or science from an institution of recognized standing or who have received the M.D. degree from a Class A medical school with at least 2 years of college training before entering the medical school.

University of Michigan

M.S. in P.H., D.P.H.—Candidates for graduate degree in public health, for the master's degree, M.S. in P.H., as well as for the doctorate, D.P.H., are obviously better equipped for their work if they already hold the degree of Doctor of Medicine from an accredited school. Holders of a baccalaureate degree from an accredited college, however, may be admitted to study for the master's degree or even, in exceptional cases, for the doctor's degree.

University of Toronto

D.P.H.—Graduate in medicine of this university or some other university recognized for this purpose by the Senate.

University of Western Ontario

D.P.H.—Graduate of approved School of Medicine.

Wayne University College of Medicine

Dr.P.H.—Graduate of Class A medical college. Applicants who have received the Degree of Doctor of Medicine since 1921 will be required to present, in addition to the diploma from the medical school, evidence of 2 years of academic instruction obtained in an approved school of arts and sciences.

Yale University

M.P.H.—Graduate in arts or sciences from a college or technical school of high standing or 2 years' study in a medical school of high standing.

Dr.P.H.—Graduate in medicine from a medical school of high standing.

* In exceptional circumstances, students without a college degree may, by special action of the Advisory Board, be accepted as candidates for the degree Sc.M. or Sc.D., provided they furnish satisfactory evidence of preparation fully equivalent to that usually represented by an academic degree, and of unusual accomplishment.

Foundations Appropriating Funds for Medicine and Public Health in 1934*

THE following Foundations made grants in excess of $5,000 for Medicine and Public Health:

American Foundation for Mental Hygiene	$ 10,033
Brez Foundation	14,250
Buhl Foundation	23,369
Carnegie Corp. of New York	91,000
Carnegie Institution of Washington	30,354
Chicago Community Chest	25,871
Children's Fund of Michigan	322,744
Cleveland Foundation	93,244
Committee of the Permanent Charity Fund	78,358
Commonwealth Fund	1,212,272
Detroit Community Chest	10,532
General Education Board	590,134
Murry and Leonie Guggenheim Foundation	90,000
Indianapolis Foundation	18,515
International Cancer Research Foundation	133,439
W. K. Kellogg Foundation	199,413
Lucius N. Littauer Foundation	13,615
Josiah Macy, Jr. Foundation	55,344
John and Mary R. Markle Foundation	41,774
Milbank Memorial Fund	124,838
New York Community Chest	70,091
New York Foundation	57,065
Horace H. and Mary A. Rackham Fund	98,700
A. C. Ratshesky Foundation	5,800
Rhode Island Foundation	5,255
Rockefeller Foundation	5,518,487
Julius Rosenwald Fund	121,450
Russell Sage Foundation	6,100
Thomas Thompson Trust	20,000
Amherst H. Wilder Charity	23,792
Williamsport Foundation	7,814
Youngstown Foundation	10,300

* From *American Foundations and Their Fields*, Twentieth Century Fund, Inc., New York, N. Y.

Directory of State, City, and Full-time County Health Officers of the United States

State Health Officers

(As of February 1, 1936)

The United States Public Health Service list published in 1935 is the basis of the following. The variations represent later information received by the American Public Health Association through correspondence.

State	*Name of Health Officer*	*Post Office*	*Official Title*
Alabama	**J. N. Baker, M.D.	Montgomery	State Health Officer
Arizona	*G. C. Truman, M.D.	Phoenix	State Supt. of Public Health
Arkansas	*William B. Grayson, M.D.	Little Rock	State Health Officer
California	Dr. W. M. Dickie	Sacramento	Director, State Dept. of Health
Colorado	*M. Flint Haralson, M.D.	Denver	Acting Health Officer
Connecticut	**Stanley H. Osborn, M.D., C.P.H.	Hartford	Commissioner of Health
Delaware	*Arthur C. Jost, M.D.	Dover	Executive Secy., State Board of Health
Florida	W. A. McPhaul, M.D.	Jacksonville	State Health Officer
Georgia	*T. F. Abercrombie, M.D.	Atlanta	Commissioner of Health
Idaho	Lewis Williams	Boise	Commissioner of Public Welfare
Illinois	*Frank J. Jirka, M.D.	Springfield	Director of Public Health
Indiana	*V. K. Harvey, M.D.	Indianapolis	State Health Commissioner
Iowa	*W. L. Bierring, M.D.	Des Moines	" " "
Kansas	**Earle G. Brown, M.D.	Topeka	Secy., State Board of Health
Kentucky	**A. T. McCormack, M.D., D.P.H.	Louisville	State Health Officer
Louisiana	*Joseph A. O'Hara, M.D.	New Orleans	Pres., State Dept. of Health
Maine	*George H. Coombs, M.D.	Augusta	State Commissioner of Health
Maryland	**Robert H. Riley, M.D.	Baltimore	Director of Health
Massachusetts	*Henry D. Chadwick, M.D.	Boston	State Com. of Public Health
Michigan	**C. C. Slemons, M.D.	Lansing	State Health Commissioner
Minnesota	**A. J. Chesley, M.D.	St. Paul	Secy. and Executive Officer, State Board of Health
Mississippi	**F. J. Underwood, M.D.	Jackson	Executive Officer, Board of Health
Missouri	*E. T. McGaugh, M.D.	Jefferson City	State Health Commissioner
Montana	**W. F. Cogswell, M.D.	Helena	Health Officer
Nebraska	*Philip H. Bartholomew, M.D.	Lincoln	State Health Officer
Nevada	**Edward E. Hamer, M.D.	Carson City	" " "
New Hampshire	**Charles Duncan, M.D.	Concord	" " "
New Jersey	*J. Lynn Mahaffey, M.D.	Trenton	Director of Health, State Dept. of Health
New Mexico	**J. Rosslyn Earp, Dr.P.H.	Santa Fe	State Director of Public Health
New York	**Thomas Parran, Jr., M.D.	Albany	State Commissioner of Health
North Carolina	Carl V. Reynolds, M.D.	Raleigh	Acting State Health Officer
North Dakota	*Maysil M. Williams, M.D.	Bismarck	State Health Officer
Ohio	*Walter H. Hartung, M.D.	Columbus	Director of Health
Oklahoma	*Charles M. Pearce, M.D.	Oklahoma City	State Health Officer
Oregon	**Frederick D. Stricker, M.D.	Portland	" " "
Pennsylvania	Martha Edith MacBride-Dexter, M.D.	Harrisburg	Secretary of Health
Rhode Island	*E. A. McLaughlin, M.D.	Providence	State Health Officer
South Carolina	**James A. Hayne, M.D.	Columbia	" " "
South Dakota	*Park B. Jenkins, M.D.	Pierre	State Superintendent of Health
Tennessee	**W. C. Williams, M.D.	Nashville	Commissioner of Health
Texas	**John William Brown, M.D.	Austin	State Health Officer
Utah	*J. Louis Jones, M.D.	Salt Lake City	State Health Commissioner
Vermont	Charles F. Dalton, M.D.	Burlington	State Health Officer
Virginia	**I. C. Riggin, M.D.	Richmond	State Health Commissioner
Washington	**Erval R. Coffey, M.D.	Seattle	State Director of Health
West Virginia	*A. E. McClue, M.D.	Charleston	State Commissioner of Health
Wisconsin	**C. A. Harper, M.D.	Madison	State Health Officer
Wyoming	G. M. Anderson, M.D.	Cheyenne	" " "

Territorial and Insular Health Officers

Alaska	*Walter W. Council, M.D.	Juneau	Commissioner of Health
Hawaii	*F. E. Trotter, M.D.	Honolulu	President, Board of Health
Panama, C. Z.	*Col. O. G. Brown, M.D., Dr.P.H.	Balboa Heights	Chief Health Officer, Medical Corps, U. S. Army
Philippine Islands	*Jose Fabella, M.D.	Manila	Director of Health and Welfare
Puerto Rico	**E. Garrido Morales, M.D.	San Juan	Commissioner of Health
Virgin Islands	Knud Knud-Hansen, M.D.	St. Thomas	Commissioner of Public Health

* Member, A.P.H.A. ** Fellow, A.P.H.A.

City Health Officers (967)

(As of February 1, 1936)

The United States Public Health Service list published in 1935 is the basis of the following. The variations represent later information received by the American Public Health Association through correspondence.

State and City	*Name of Health Officer*	*Official Title*
Alabama		
Anniston	Dr. George A. Cryer	County Health Officer
Bessemer	*J. D. Dowling, M.D.	" " "
Birmingham	*J. D. Dowling, M.D.	" " "
Decatur	*Lee Roy Murphree, M.D.	" " "
Dothan	*F. G. Granger, M.D.	" " "
Fairfield	*J. D. Dowling, M.D.	Health Officer
Florence	*W. D. Hubbard, M.D.	County Health Officer
Gadsden	*C. L. Murphree, M.D.	" " "
Huntsville	Dr. W. C. Hatchett	" " "
Mobile	*O. L. Chason, M.D.	" " "
Montgomery	*J. L. Bowman, M.D.	County & City Health Officer
Phenix City	Dr. M. L. Shaddix	Health Officer
Selma	*L. T. Lee, M.D.	County Health Officer
Tuscaloosa	Dr. A. A. Kirk	" " "
Arizona		
Phoenix	Dr. R. W. Hussong	City Health Officer
Tucson	*Lewis H. Howard, M.D.	" " "
Arkansas		
Blytheville	Dr. I. R. Johnson	" " "
El Dorado	Dr. F. O. Mahony	" " "
Fort Smith	*James E. Johnson, M.D.	District Health Officer
Hot Springs	J. F. Merritt, M.D.	City Health Officer
Jonesboro	Dr. R. C. Shanlever	" " "
Little Rock	V. T. Webb, M.D.	" " "
North Little Rock	Dr. Val L. Eason	" " "
Pine Bluff	Dr. W. H. Bruce	Director of Health
Texarkana	Dr. Harry E. Murry	City Health Officer
California		
Alameda	*F. B. Galbraith, M.D.	Health Officer
Alhambra	**S. J. Stewart, M.D.	Medical Director
Anaheim	*K. H. Sutherland, M.D.	County Health Officer
Bakersfield	**Peter Joseph Cuneo, M.D.	Health Officer
Berkeley	**Frank L. Kelly, M.D.	" "
Beverly Hills	*Charles F. Nelson, M.D.	" "
Brawley	J. L. Parker, M.D.	" "
Burbank	T. H. Ransom, M.D.	" "
Burlingame	Matthew F. Desmond, M.D.	" "
Compton	*F. E. Estes, M.D.	" "
Eureka	*William J. Quinn, M.D.	" "
Fresno	C. Mathewson, M.D.	City Health Officer
Fullerton	*K. H. Sutherland, M.D.	County Health Officer
Glendale	*F. A. Wilmot, M.D.	District Health Officer
Huntington Park	*George M. Malkin, M.D.	" " "
Inglewood	*J. W. Robinson, M.D.	Deputy Health Officer
Long Beach	**G. E. McDonald, M.D.	City Health Officer
Los Angeles	**George Parrish, M.D.	Health Officer
Modesto	*E. F. Reamer, M.D.	County Health Officer
Monrovia	**J. M. Furstman, M.D.	County Health Officer
Oakland	*Nolton N. Ashley, M.D.	Health Officer
Ontario	C. L. Emmons, M.D.	" "
Palo Alto	**Louis Olsen	" "
Pasadena	**W. L. Halverson, M.D.	" "
Pomona	*M. U. Stoneman, M.D.	District Health Officer
Redlands	Dr. F. H. Folkins	Health Officer
Richmond	Charles Robert Blake, M.D.	City Health Commissioner
Riverside	*W. A. Jones, M.D.	Commissioner of Health
Sacramento	**Herbert F. True, M.D.	City Health Officer
Salinas	*Marie Fidel	Health Officer
San Bernardino	Dr. G. Stirling Landon	City Health Officer
San Diego	*Alex M. Lesem, M.D.	Health Officer

* Member, A.P.H.A. ** Fellow, A.P.H.A.

State and City	Name of Health Officer	Official Title
California (Cont.)		
San Francisco	**J. C. Geiger, M.D.	Health Officer
San Jose	*Henry C. Brown, M.D.	" "
San Leandro	**I. O. Church, M.D.	District Health Officer
San Mateo	Dr. J. R. Warberton	City Health Officer
Santa Ana	*K. H. Sutherland, M.D.	County Health Officer
Santa Barbara	*C. T. Roome, M.D.	Health Officer
Santa Cruz	John T. Harrington, M.D.	City Health Officer
Santa Monica	**F. G. Crandall, M.D.	District Health Officer
Santa Rosa	E. J. Helgren	Health Officer
South Gate	Hal E. Hazel	" "
South Pasadena	*Albert J. Supple, M.D.	" "
Stockton	**John J. Sippy, M.D.	District Health Officer
Vallejo	E. A. Peterson, M.D.	Health Officer
Ventura	Dr. J. A. De Serpa	" "
Whittier	*R. L. Kaufman, M.D.	District Health Officer
Colorado		
Boulder	*H. L. Morency, M.D.V.	Health Officer
Colorado Springs	**Omer R. Gillett, M.D.	City Health Officer
Denver	Dr. Fred W. Bailey	Manager of Health and Charity
Fort Collins	Dr. T. C. Taylor	Health Officer
Grand Junction	Dr. E. H. Munro	" "
Greeley	Dr. W. A. Schoen	City Health Officer
Pueblo	**W. E. Buck, M.D.	Chief, Dept. of Health
Trinidad	Dr. B. M. Cowley	City Physician
Connecticut		
Ansonia	Dr. L. Howard Wilmot	Health Officer
Bridgeport	*Richard O'B. Shea, M.D.	" "
Bristol	*Benjamin B. Robbins, M.D.	City Health Officer
Danbury	Dr. Felix F. Tomaino	Health Officer
Derby	Thomas F. Plunkett, M.D.	" "
East Hartford	Dr. Francis W. Becker	" "
Enfield	Dr. Frank F. Simonton	" "
Fairfield	**Lawrence E. Poole, M.D.	Health Officer & School Physician
Groton	Dr. Frank W. Hewes	Health Officer
Hamden	*George H. Joslin, M.D.	" "
Hartford	*T. F. O'Brien, M.D.	" "
Manchester	*D. C. Y. Moore, M.D.	Chairman, Board of Health
Meriden	*Dr. Michael J. Sullivan	Health Officer
Middletown	John G. Mountain, M.D.	" "
Milford	.....	" "
Naugatuck	.....	Town Health Officer
New Britain	**Louis J. Dumont, M.D.	Supt. of Health
New Haven	*Joseph I. Linde, M.D.	Health Officer
New London	*B. N. Pennell, D.V.S.	" "
Norwalk	Robert E. Perdue, M.D.	" "
Norwich	*Harrison Gray, M.D.	City Health Officer
Shelton	Dr. Edwin J. Finn	Health Officer
Stamford	*Raymond D. Fear, M.D.	Health Commissioner
Stonington	Dr. William D. Veal	Health Officer
Stratford	DeRuyter Howland, M.D.	" "
Torrington	Dr. Elias Pratt	" "
Wallingford	.....	" "
Waterbury	*Edward J. Godfrey, M.D.	City Health Officer
West Hartford	*Harry B. Smith, M.D., M.P.H.	Health Officer
Willimantic	Nathan Spector, M.D.	" "
Delaware		
Wilmington	Dr. James W. Butler	Secretary, Board of Health
District of Columbia		
Washington	**George C. Ruhland, M.D.	Commissioner of Health
Florida		
Daytona Beach	Dr. Simon Reed	Health Officer
Gainesville	*W. W. Lassiter, M.D.	" "
Jacksonville	**Noble A. Upchurch, M.D.	City Health Officer
Key West	H. C. Galey, M.D.	" " "
Lakeland	Dr. J. D. Griffin	City Physician & Health Officer
Miami	*George N. MacDonell, M.D.	Director of Public Health
Orlando	Dr. C. D. Christ	City Health Officer

* Member, A.P.H.A. ** Fellow, A.P.H.A.

State and City	Name of Health Officer	Official Title
Florida (Cont.)		
Pensacola	.....	Health Officer
St. Augustine	Dr. H. E. White	" "
St. Petersburg	Dr. Claude B. Wright	City Physician
Sanford	J. N. Tolar, M.D.	Health Officer
Tallahassee	Dr. L. J. Graves	" "
Tampa	*J. R. McEachern, M.D.	City Health Officer
West Palm Beach	W. E. Van Landingham, M.D.	" " "
Georgia		
Albany	Hugo Robinson, M.D.	Health Commissioner
Athens	*W. W. Brown, M.D.	" "
Atlanta	**J. P. Kennedy, M.D.	City Health Officer
Augusta	**Henry G. Callison, M.D.	City & County Health Officer
Brunswick	**M. E. Winchester, M.D.	Commissioner of Health
Columbus	Dr. W. E. Mayher	Health Officer
Decatur	Dr. J. R. Evans	" "
Griffin	Dr. William C. Humphries	Commissioner of Health
La Grange	S. C. Rutland, M.D.	" " "
Macon	*J. D. Applewhite, M.D.	City & County Health Officer
Rome	B. V. Elmore, M.D.	Commissioner of Health
Savannah	**Victor H. Bassett, M.D.	Health Officer
Thomasville	Dr. James R. Dykes	" "
Valdosta	Gordon T. Crozier, M.D.	City Health Officer
Waycross	*George E. Atwood, M.D., Dr.P.H.	Commissioner of Health
Idaho		
Boise	C. K. Macay	Health Officer
Pocatello	*E. O. Leonard	Sanitary Inspector
Illinois		
Alton	Dr. William S. McGinnis	Health Commissioner
Aurora	*George W. Haan, M.D.	" "
Belleville	Dr. R. C. Heiligenstein	Health Officer
Berwyn	*Edward J. Farrell, M.D.	Health Director
Bloomington	Dr. B. Markowitz	" "
Blue Island	*L. A. Burkhart	Health Commissioner
Brookfield	Harriet L. Hockendorf	" "
Cairo	C. L. Weber, M.D.	Health Officer & City Physician
Calumet City	E. S. O'Brien, M.D.	Health Officer
Canton	Dr. J. C. Simmons	City Physician
Centralia	Dr. J. M. Haney	City Health Officer
Champaign	Dr. George Appelle	Health Officer
Chicago	**Herman N. Bundesen, M.D.	Pres., Board of Health
Chicago Heights	Dr. Ira C. Harman	Health Commissioner
Cicero	Dr. Frank J. Pokorney	" "
Danville	Dr. C. M. Cook	" "
Decatur	*W. M. Talbert, M.D.	Medical Director, Dept. of Public Health and Safety
East Moline	J. H. Fowler, M.D.	Commissioner of Health
East St. Louis	Albert P. Lauman	" " "
Elgin	Dr. A. L. Mann	Executive Officer, Dept. of Health
Elmhurst	Dr. A. L. Mathias	Health Commissioner
Elmwood Park	*Laura Arney	Health Officer
Evanston	*John H. Pollard, M.D.	Commissioner of Health
Forest Park	Dr. George Baumgartner	" " "
Freeport	Dr. R. J. Burns	" " "
Galesburg	E. D. Wing, M.D.	Health Officer
Granite City	Dr. A. M. Jennings	" "
Harrisburg	Charles Walden, M.D.	City Physician
Harvey	M. R. Morse, M.D.	Health Officer
Highland Park	Dr. D. E. Rossiter	President, Board of Health
Jacksonville	Dr. Friedrich Engelbach	Health Officer
Joliet	Dr. E. J. Higgins	Commissioner of Health
Kankakee	Dr. Joseph A. Guertin	City Health Officer
Kewanee	Dr. C. P. White	Commissioner of Health
LaGrange	Dr. T. C. McDougal	Village Health Officer
LaSalle	*Arlington Ailes, M.D.	Health Commissioner
Lincoln	Claude Applegate	Health Officer

* Member, A.P.H.A. ** Fellow, A.P.H.A.

State and City	*Name of Health Officer*	*Official Title*
Illinois (Cont.)		
Mattoon	Dr. Lowell A. Neal	City Health Officer
Maywood	Dr. John Peters	Health Commissioner
Melrose Park	Dr. E. G. Brust	Health Officer
Moline	Dr. C. C. Ellis	" "
Mt. Vernon	Dr. Runyon Irvin	City Physician
Oak Park	*Frank S. Needham, M.D.	Commissioner of Health
Ottawa	E. P. Hathaway, M.D.	City Physician
Park Ridge	Dr. M. W. Caveney	Health Officer
Pekin	Dr. C. G. Muehlman	City Health Officer
Peoria	*Sumner M. Miller, M.D.	Health Commissioner
Quincy	*H. O. Collins, M.D.	Public Health Officer
Rockford	*Norman C. Bullock, M.D.	Commissioner of Health
Rock Island	Dr. H. W. Shuman	City Physician
Springfield	*H. H. Tuttle, M.D.	Superintendent of Health
Sterling	Dr. H. M. Jacobs	Health Officer
Streator	Dr. Theresa K. Jennings	President, Board of Health
Urbana	Dr. L. M. T. Stilwell	Chairman, Board of Health
Waukegan	Dr. H. Floyd Cannon	City Health Officer
West Frankfort	William T. Fife	" " "
Wilmette	Dr. Martin H. Seifert	Commissioner of Health
Winnetka	*H. A. Orvis, M.D.	Health Officer
Indiana		
Anderson	Dr. G. E. Metcalf	Secretary, Board of Health
Bedford	Charles H. Blackburn	Health Officer
Bloomington	Dr. C. E. Holland	" "
Connersville	Herman W. Smelser, M.D.	" "
Crawfordsville	Fred N. Daugherty, M.D.	Secretary, Board of Health
East Chicago	Dr. Joseph A. Teegarden	" " " "
Elkhart	I. J. Markel, M.D.	" " " "
Elwood	Dr. Frank V. Newcomer	" " " "
Evansville	L. E. Fritsch, M.D.	" " " "
Fort Wayne	Dr. Karl C. Eberly	Health Commissioner
Frankfort	Dr. A. G. Chittick	Secretary, Board of Health
Gary	Walter M. Behn, M.D.	" " " "
Goshen	G. A. Whippy, M.D.	Health Officer
Hammond	Dr. H. G. Cole	Health Commissioner
Huntington	R. F. Frost, M.D.	Secretary, Board of Health
Indianapolis	Herman G. Morgan, M.D.	" " " "
Jeffersonville	Samuel L. Adair, M.D.	Health Officer
Kokomo	.....	Secretary, Board of Health
LaFayette	M. M. Lairy, M.D.	" " " "
LaPorte	J. N. Kelly, M.D.	Health Officer
Logansport	Louis P. Deuner	Health Inspector
Marion	L. H. Eshleman, M.D.	Secretary, Board of Health
Michigan City	Dr. L. M. Robrock	Health Officer
Mishawaka	Lyman Swanger	Secretary, Board of Health
Muncie	Dr. J. H. Williams	" " " "
New Albany	A. I. McKamy, Ph.D., M.D.	" " " "
Newcastle	Walter M. Stout, M.D.	" " " "
Peru	W. H. Wagoner, M.D.	" " " "
Richmond	M. F. Johnston, M.D.	Health Commissioner
Shelbyville	Walter C. McFadden, M.D.	Health Officer
South Bend	*F. R. Nicholas Carter, M.D.	" "
Terre Haute	Dr. Amos H. Caffee	Secretary, Board of Health
Vincennes	R. S. Moore, M.D.	" " " "
Whiting	Dr. J. A. McCarthy	City Health Officer
Iowa		
Ames	Dr. B. D. Atchley	City Health Officer
Boone	William Woodburn, M.D.	Health Officer
Burlington	Dr. Arthur C. Schach	City Physician
Cedar Rapids	James Yanda	City Health Officer
Clinton	Dr. Leslie K. Fenlon	" " "
Council Bluffs	Dr. J. M. Moskovitz	" " "
Davenport	*A. B. Kuhl, Jr., M.D.	Health Officer
Des Moines	*Harry E. Ransom, M.D.	Health Commissioner
Dubuque	W. J. Connell, M.D.	Director of Health
Fort Dodge	Dr. J. E. Galvin	City Physician
Fort Madison	Harold F. Noble, M.D.	" "

* Member, A.P.H.A. ** Fellow, A.P.H.A.

State and City	Name of Health Officer	Official Title
Iowa (Cont.)		
Iowa City	Dr. Isom A. Rankin	Health Officer
Keokuk	Dr. Charles A. Dimond	" "
Marshalltown	Dr. R. S. Grossman	" "
Mason City	Dr. C. M. Franchere	City Health Officer
Muscatine	R. M. Arey, M.D.	" " "
Newton	Dr. M. R. Hammer	City Physician
Oskaloosa	Dr. Oscar J. DuBois	Health Officer
Ottumwa	Dr. William Herrick	City Health Officer
Sioux City	*W. S. Petty, M.D.	Field Agent, U.S.P.H.S.
Waterloo	J. E. Ridenour, M.D.	Health Officer
Kansas		
Arkansas City	Dr. P. F. Theis	City Health Officer
Atchison	Dr. William K. Fast	County Health Officer
Chanute	Dr. R. A. Light	City Health Officer
Coffeyville	Dr. P. S. Townsend	" " "
Dodge City	C. L. Hooper	Health Officer
Eldorado	Jack Loman	Sanitary Inspector
Emporia	Dr. C. H. Munger	County Health Officer
Fort Scott	C. L. Mosley, M.D.	City Health Officer
Hutchinson	G. R. Walker, M.D.	City Physician
Independence	.....	City Health Officer
Kansas City	Dr. H. W. Kassel	Director of Health
Lawrence	*James M. Mott, M.D.	Supt. of Public Health
Leavenworth	Dr. A. R. Adams	City Health Officer
Manhattan	Dr. Darrell Evans	Health Officer
Newton	Dr. M. C. Martin	" "
Parsons	Dr. L. A. Proctor	City Health Officer
Pittsburg	Dr. C. Mart Montes	" " "
Salina	Dr. W. R. Dillingham	" " "
Topeka	Dr. F. P. Helm	Secretary, Board of Health
Wichita	*J. E. Wolfe, A.B., M.D.	Director, Public Welfare
Kentucky		
Ashland	R. D. Higgins, M.D.	Health Officer
Bowling Green	*George M. Wells, M.D.	" "
Covington	Dr. Theodore Sallee	City Health Officer
Fort Thomas	Dr. Frank H. Southgate	Health Officer
Frankfort	Dr. R. M. Coblin	City Health Officer
Henderson	Dr. J. L. Tauner	County Health Officer
Hopkinsville	Philip E. Haynes, M.D.	City Health Officer
Lexington	Dr. Charles M. Moore	Health Officer
Louisville	*Hugh R. Leavell, M.D.	Health Director
Middlesboro	M. D. Hoskins, M.D.	County Health Officer
Newport	John Todd, M.D.	Health Officer
Owensboro	Dr. A. L. Kincheloe	County Health Officer
Paducah	Dr. R. W. Robertson	City Health Officer
Louisiana		
Alexandria	Dr. R. B. Wallace	President, Board of Health
Baton Rouge	Thomas J. McHugh, M.D.	City Health Officer
Bogalusa	Dr. J. H. Slaughter	Health Officer
Lafayette	Dr. G. A. Martin	City Physician
Lake Charles	Dr. W. P. Bordelon	President, Board of Health
Monroe	Henry Haas	Supt., City Sanitary Dept.
New Orleans	*James M. Batchelor, M.D.	Superintendent of Public Health
Shreveport	*W. J. Sandidge, M.D.	" " " "
Maine		
Auburn	E. Leathers, M.D.	Health Officer
Augusta	*George A. Coombs, M.D.	" "
Bangor	Harry D. McNell, M.D.	" "
Bath	Dr. Joseph I. Smith	Health Officer & Milk Inspector
Biddeford	John W. Mahoney	Health Officer
Lewiston	*R. J. Wiseman, Jr., M.D.	" "
Portland	**Thomas Tetreau, M.D.	" "
Sanford	*William H. Kelly, M.D.	Local Health Officer
South Portland	Dr. Roderick L. Huntress	Health Officer
Waterville	Arthur R. Daviau, M.D.	" "
Westbrook	P. H. Welch	" "

* Member, A.P.H.A. ** Fellow, A.P.H.A.

State and City	Name of Health Officer	Official Title
Maryland		
Annapolis	Dr. James J. Murphy	City Health Officer
Baltimore	**Huntington Williams, M.D.	Commissioner of Health
Cumberland	Harvey H. Weiss	Health Officer & Registrar
Frederick	*E. C. Kefauver, M.D.	Health Officer
Hagerstown	Dr. W. R. Cameron	County Health Officer
Salisbury	*Seth H. Hurdle, M.D.	" " "
Massachusetts		
Adams	J. F. McLaughlin, M.D.	Chairman, Board of Health
Amesbury	Clarence S. Morse	Agent, Board of Health
Arlington	William H. Bradley	" " " "
Athol	Marion B. Sibley, M.D.	Secretary, Board of Health
Attleboro	*William O. Hewitt, M.D.	Health Officer
Belmont	*Thomas F. Harris	Agent, Board of Health
Beverly	Alonzo O. Woodbury	" " " "
Boston	W. B. Keeler, M.D.	Health Commissioner
Braintree	Mortimer N. Peck	Agent, Board of Health
Brockton	*David B. Tuholski, M.D.	Health Officer
Brookline	**Francis P. Denny, M.D.	" "
Cambridge	Dr. Simon B. Kelleher	Medical Inspector
Chelsea	John F. Welch	Health Officer
Chicopee	.....	Agent, Board of Health
Clinton	Frederick E. Murphy	" " " "
Danvers	Hogo Nappe, R.N.	Health Officer
Dedham	Thomas J. Breman	Health Inspector
Easthampton	Clemence C. Buckner	Agent, Board of Health
Everett	William F. Hogan	" " " "
Fairhaven	F. Fred Delano	Executive Officer
Fall River	*Ernest M. Morris, M.D.	Health Commissioner
Fitchburg	Fred R. Brigham	Agent, Board of Health
Framingham	David Moxon, C.P.H.	" " " "
Gardner	William P. O'Donnell	" " " "
Gloucester	Dr. P. E. Curley	Sanitary Inspector
Greenfield	George P. Moore	Agent, Board of Health
Haverhill	*Fred W. Morse	" " " "
Holyoke	Dr. Daniel P. Harnett	Health Officer
Lawrence	Daniel J. Costello	Clerk, Board of Health
Leominster	Hugh E. Crain	Chairman, Board of Health
Lowell	*John J. McNamara, M.D.	Agent, Board of Health
Lynn	*James A. Dumas, M.D.	Commissioner of Public Health
Malden	Mary C. Welsh	Agent, Board of Health
Marlborough	John J. Cassidy	" " " "
Medford	William M. Lanigan, M.D.	Medical Inspector
Melrose	Clarence P. Holden, M.D.	Chairman, Board of Health
Methuen	Dr. John Oddy	Board of Health Physician
Milford	Clifton Tyler	Secretary, Board of Health
Milton	Paul W. Kimball, M.D.	Agent, Board of Health
Natick	Dr. Charles D. Colford	" " " "
Needham	*G. D. Buckner	Health Officer
New Bedford	**William G. Kirschbaum	Agent & Executive Officer
Newburyport	*W. N. O'Brien, Ph.D.	Agent, Board of Health
Newton	*Harold D. Chope, M.D.	Health Officer
North Adams	Douglas W. Hyde, S.E.	Agent, Board of Health
North Attleboro	Dr. Michael E. Vance	Health Officer
Northampton	George R. Turner	Agent, Board of Health
Norwood	John A. Shannon	" " " "
Peabody	*Percy F. Murray	" " " "
Pittsfield	*Willys M. Monroe, M.D.	City Health Officer
Plymouth	Almeda Chandler	.....
Quincy	Dr. Richard M. Ash	Health Commissioner
Revere	Dr. Frank Sandler	Chairman, Board of Health
Salem	**John J. McGrath	Agent, Board of Health
Saugus	Henry C. Westendarp	Chairman, Board of Health
Somerville	Frank L. Morse, M.D.	Medical Inspector
Southbridge	Albert R. Brown	Agent, Board of Health
Springfield	*L. J. Smith, M.D.	City Health Officer
Stoneham	*George A. Hinchcliffe	Health Officer
Swampscott	Clarence W. Horton	" "
Taunton	*John J. McNamara, M.D.	Chairman, Board of Health
Wakefield	David Taggart	Health Officer

* Member, A.P.H.A. ** Fellow, A.P.H.A.

State and City	*Name of Health Officer*	*Official Title*
Massachusetts (Cont.)		
Waltham	Joseph T. Mulcahy	Director of Public Welfare
Watertown	Fred W. Bodge	Health Officer
Webster	Dr. Daniel Casey	Chairman, Board of Health
Wellesley	**Prof. C. M. Hilliard	Health Officer
Westfield	Dr. Robert M. Marr	Chairman, Board of Health
West Springfield	John J. Lysaght	Agent, Board of Health
Weymouth	F. L. Doucett, M.D.	Clerk, Board of Health
Winchester	*Maurice Dineen	Agent, Board of Health
Winthrop	William D. Childress	" " " "
Woburn	Edward T. Gorman	Health Officer
Worcester	*Peter O. Shea, M.D.	Director, Board of Health
Michigan		
Adrian	Dr. W. S. Mackenzie	Health Officer & City Physician
Alpena	Dr. F. J. O'Donnell	Health Officer
Ann Arbor	.....	" "
Battle Creek	A. A. Hoyt, M.D.	" "
Bay City	*George W. Moore, M.D.	City Physician
Benton Harbor	E. R. Taylor, M.D.	Director of Public Health
Dearborn	*C. A. Christensen, M.D.	Commissioner of Health
Detroit	**Henry F. Vaughan, Dr.P.H.	" " "
Ecorse	L. H. Van Becelaere, M.D.	Health Officer
Escanaba	Dr. Harry J. Defnet	" "
Ferndale	Dr. Willard G. Beattie	" "
Flint	*Leslie Lambert, M.D.	" "
Grand Rapids	**John L. Lavan, M.D.	" "
Grosse Pointe	*Benjamin H. Warren, M.D.	" "
Hamtramck	Dr. Peter E. Bolewicki	Health Commissioner
Highland Park	Dr. George M. Livingston	Health Officer
Holland	William Westrate, M.D.	" "
Iron Mountain	James L. Browning, M.D.	" "
Ironwood	*C. C. Urquhart, M.D.	City Health Officer
Jackson	.....	Health Officer
Kalamazoo	*I. W. Brown, M.D.	Director of Health
Lansing	*E. R. Vanderslice, M.D.	Health Director
Lincoln Park	Dr. H. K. Butterworth	Health Officer
Marquette	*F. McD. Harkin, M.D.	" "
Menominee	Dr. John T. Kaye	" "
Monroe	Dr. William F. Acker	" "
Mount Clemens	W. S. Kane, M.D.	.....
Muskegon	Dr. M. E. Stone	Health Officer
Muskegon Heights	Dr. O. M. LaCore	" "
Niles	Dr. Lawrence M. Rutz	" "
Owosso	W. E. Ward, M.D.	" "
Pontiac	*C. A. Neafie, M.D.	Director of Public Health
Port Huron	A. L. Callery, M.D.	Health Officer
River Rouge	Dr. Claud Smith	" "
Royal Oak	Dr. Donald A. Cameron	City Health Officer
Saginaw	*Frank A. Poole, M.D.	Health Officer
Sault Ste. Marie	*E. A. Cornell, M.D.	" "
Traverse City	G. A. Holliday, M.D.	" "
Wyandotte	*Earl H. Engel, M.D.	" "
Ypsilanti	Dr. B. M. Harris	City Health Officer
Minnesota		
Albert Lea	Dr. Donald S. Branham	Health Inspector
Austin	Dr. Peter A. Lommen	Chairman, Board of Health
Brainerd	Dr. V. E. Quanstrom	City Health Officer
Duluth	*M. McC. Fischer, M.D.	Health Officer
Faribault	*Frederick U. Davis, M.D.	Health Commissioner
Hibbing	Dr. C. N. Harris	Chairman, Board of Health
Mankato	Dr. W. A. Beach	Health Commissioner
Minneapolis	*F. E. Harrington, M.D.	" "
Rochester	*C. H. Mayo, M.D.	Health Officer
St. Cloud	Dr. H. W. Goehrs	City Physician
St. Paul	Dr. R. B. J. Schoch	Health Officer
South St. Paul	Dr. O. S. Ely	" "
Virginia	Dr. J. Arnold Malmstrom	" "
Winona	*William V. Lindsay, M.D.	" "

* Member, A.P.H.A. ** Fellow, A.P.H.A.

State and City	*Name of Health Officer*	*Official Title*
Mississippi		
Biloxi	G. F. Carroll, M.D.	Health Officer
Clarksdale	*N. C. Knight, M.D.	Director, County Health Dept.
Columbus	Dr. C. E. Lehmberg	County Health Officer
Greenville	*John W. Shackleford, M.D.	City Health Officer
Greenwood	*L. A. Barnett, M.D.	Health Officer
Gulfport	Daniel J. Williams, M.D.	" "
Hattiesburg	*B. D. Blackwelder, M.D.	County Health Officer
Jackson	W. E. Noblin, M.D.	Director, County Health Unit
Laurel	Dr. L. B. Beech	City Health Officer
McComb	**T. Paul Haney, Jr., M.D.	County Health Officer
Meridian	*D. V. Galloway, M.D.	Director, County Health Dept.
Natchez	*A. R. Perry, M.D.	" " " "
Vicksburg	*F. Michael Smith, M.D.	" " " "
Missouri		
Cape Girardeau	Lee Slagle	Sanitary Inspector
Columbia	W. A. Norris, M.D.	City Health Commissioner
Hannibal	*Eugene M. Lucke, M.D.	Field Agent
Independence	F. L. Cook, M.D.	City Physician
Jefferson City	Dr. James G. Bruce	"
Joplin	Dr. A. Benson Clark	Commissioner of Health & Sanitation
Kansas City	.....	Director of Health
Maplewood	Dr. Pierre M. Brossard	Health Officer
Moberly	Dr. C. C. Smith	City Health Officer
St. Charles	Dr. L. E. Belding	City Physician
St. Joseph	A. J. Smith, M.D.	City Health Officer
St. Louis	*Joseph F. Bredeck, M.D., D.P.H.	Health Commissioner
Sedalia	J. H. Brooks	Sanitary Officer
Springfield	Ralph W. Langston	Commissioner of Health & Sanitation
University City	*O. P. Hampton, Jr., M.D.	Health Officer
Webster Groves	*Carl C. Irick, M.D.	Health Commissioner
Montana		
Anaconda	Dr. John J. Malee	City Physician
Billings	*E. G. Balsam, M.D.	City Health Officer
Butte	Joseph J. Kane, M.D.	" " "
Great Falls	**Frank L. Watkins, M.D.	Field Agent, U.S.P.H.S.
Helena	Dr. W. M. Copenhaver	City Health Officer
Missoula	*F. D. Pease, M.D.	Health Officer
Nebraska		
Beatrice	Dr. J. R. Leibee	City Physician
Fremont	Dr. R. T. Van Metre	Health Officer
Grand Island	W. M. Wheeler	City Engineer
Hastings	*E. J. Latta, M.D.	City Physician
Lincoln	*M. F. Arnholt, M.D.	Superintendent of Health
Norfolk	Dr. V. L. Seman	Secretary, Board of Health
North Platte	Josiah B. Redfield, M.D.	City Physician
Omaha	Dr. F. H. Kinyoun	Health Commissioner
Nevada		
Reno	A. F. Adams, M.D.	Secretary, Board of Health
New Hampshire		
Berlin	*Eli A. Marcoux, B.S.	Health Officer
Claremont	*William P. Prescott	" "
Concord	*Travis P. Burroughs, M.D.	City Health Officer
Dover	William E. Whiteley	Executive Officer, Board of Health
Keene	Dr. Arthur A. Pratt	Health Officer
Laconia	Dr. E. J. Gage	" "
Manchester	**Howard A. Streeter, M.D.	" "
Nashua	*Deering G. Smith, M.D.	Chairman, Health Dept.
Portsmouth	Dr. L. R. Hazzard	Chairman, Board of Health
Rochester	Charles E. Goodwin	Health Officer
New Jersey		
Asbury Park	**Budd H. Obert, M.D.	Health Officer
Atlantic City	**Samuel L. Salasin, M.D.	" "
Bayonne	**William W. Brooke, M.D.	" "
Belleville	Eugene T. Berry	" "
Bloomfield	*Joseph C. Saile, M.D.	" "

* Member, A.P.H.A. ** Fellow, A.P.H.A.

State and City	*Name of Health Officer*	*Official Title*
New Jersey (Cont.)		
Bridgeton	John E. Robbins	Sanitary Inspector
Burlington	Mrs. Kathryn C. Phillips	Health Officer
Camden	A. L. Stone, M.D.	Director of Public Health
Cartaret	.....	Health Officer
Cliffside Park	Fred J. Dyer	" "
Clifton	*Lester F. Meloney, M.D.	" "
Collingswood	Harold K. Eynon, M.D.	Medical Inspector
Dover	John G. Taylor	Health Officer
East Orange	**F. J. Osborne	" "
Elizabeth	*Louis J. Richards	" "
Englewood	*H. R. H. Nicholas	" "
Carfield	Charles B. Bleasby, M.D.	" "
Gloucester	J. Alonzo Beek, M.D.	" "
Hackensack	**L. Van D. Chandler	" "
Harrison	John T. McClure	" "
Hawthorne	Dr. William Missouellie	" "
Hoboken	Joseph F. X. Stack, M.D.	Commissioner of Health
Irvington	William S. Bailey	Health Officer
Jersey City	James J. Hagan, M.D.	" "
Kearny	*Amos Field, Jr.	" "
Linden	M. E. Noe	" "
Lodi	H. H. Brevoort, M.D.	Health Inspector
Long Branch	R. Clifford Errickson	" "
Maplewood	*Marie Harrison, R.N.	Health Officer
Millville	R. H. Knowles, Ph.G.	" "
Montclair	*Carl T. Pomeroy, C.P.H.	" "
Morristown	John F. Kilkenny	" "
New Brunswick	E. I. Cronk, M.D.	" "
Newark	**Charles V. Craster, M.D.	" "
Nutley	Eugene H. Sullivan	" "
Orange	*William M. Brien, M.D.	" "
Passaic	*John N. Ryan, M.D.	" "
Paterson	**F. P. Lee, M.D.	" "
Perth Amboy	*Charles S. Thompson, D.V.S.	" "
Phillipsburg	Dr. William Dana Pursel	Town Physician
Plainfield	*Andrew J. Krog	Acting Health Officer
Pleasantville	Dr. Robert M. Grier	Health Inspector
Rahway	*Fred M. Williams	Health Officer
Red Bank	William H. Lawes	Sanitary Inspector
Ridgefield	William F. Reynolds, D.V.M.	Health Officer
Ridgewood	H. H. Pettit, M.D.	" "
Roselle	*P. A. Proudfoot, M.D.	" "
Rutherford	Marine Dunn	Sanitary Inspector
South Orange	Dr. A. C. Benedict	Health Officer
South River	Dr. Abraham A. Pansy	" "
Summit	*Henry P. Dengler, M.D.	Executive Officer, Board of Health
Trenton	Alton S. Fell, M.D.	Health Officer
Union City	Grant P. Curtis, M.D.	" "
Westfield	Andrew Carney	" "
West New York	*Rudolph Kunze	Chief Inspector
West Orange	David E. Buckley	Health Officer
New Mexico		
Albuquerque	**James R. Scott, M.D.	Health Officer
Roswell	W. W. Phillipps, M.D.	County Health Officer
Santa Fe	*E. F. McIntyre, M.D.	Health Officer
New York		
Albany	*Daniel V. O'Leary, M.D.	Health Officer
Amsterdam	*P. J. Fitzgibbons, M.D.	" "
Auburn	*John W. Copeland, M.D.	" "
Batavia	Emery F. Will, M.D.	" "
Beacon	Charles B. Dugan, M.D.	" "
Binghamton	*Chalmer J. Longstreet, M.D.	" "
Buffalo	**Francis E. Fronczak, M.D.	Health Commissioner
Cohoes	Dr. E. M. Bell	City Health Officer
Corning	*Henry E. Elwood, Jr., M.D.	Health Officer
Cortland	**Merle French, M.D.	" "
Dunkirk	*Edgar Bieber, M.D.	" "
Elmira	*Reeve B. Howland, M.D.	" "

* Member, A.P.H.A. ** Fellow, A.P.H.A.

State and City	*Name of Health Officer*	*Official Title*
New York (Cont.)		
Endicott	*M. W. Welch, M.D.	Health Officer
Floral Park	*Arthur E. Goldfarb, M.D.	" "
Freeport	*William H. Runcie, M.D.	" "
Fulton	Dr. F. Edward Fox	" "
Geneva	**C. W. Grove, M.D.	" "
Glen Cove	Joseph B. Connolly, M.D.	" "
Glens Falls	Virgil D. Selleck, M.D.	" "
Gloversville	Felix L. Johnson, M.D.	" "
Hempstead	*William H. Runcie, M.D.	" "
Herkimer	James W. Graves, M.D.	" "
Hornell	*George E. Taylor, M.D.	" "
Hudson	*Louis Van Hoesen, M.D.	" "
Ithaca	*Lowell T. Genung, M.D.	" "
Jamestown	*William M. Sill, M.D.	Supt. of Public Health
Johnson City	Rollin O. Crosier, M.D.	Health Officer
Johnstown	Guy Vail Wilson, M.D.	" "
Kenmore	*E. R. Linklater, M.D.	" "
Kingston	L. E. Sanford, M.D.	" "
Lackawanna	*L. M. Michalek, M.D.	" "
Little Falls	George S. Eveleth, M.D.	" "
Lockport	Dr. G. H. Barone	City Health Officer
Lynbrook	Dr. F. M. Galloway	Health Officer
Mamaroneck	Dr. Edward M. Clark	" "
Massena	*C. E. Elkins, M.D.	" "
Middletown	*H. J. Shelley, M.D.	" "
Mount Vernon	Frank W. Shipman, M.D.	Commissioner of Health
Newburgh	Thomas J. Burke, M.D.	Health Officer
New Rochelle	*Bertrand F. Drake, M.D.	" "
New York	**John L. Rice, M.D.	Commissioner of Health
Niagara Falls	*Edward E. Gillick, M.D.	Health Officer
North Tonawanda	H. C. Lapp, M.D.	" "
Ogdensburg	*Frederick E. Clark, M.D.	" "
Olean	*Joseph P. Garen, M.D.	" "
Oneida	Dr. E. L. Finley	" "
Oneonta	Dr. George W. Augustin	" "
Ossining	Robert R. Bloom, M.D.	" "
Oswego	Dr. James E. Mansfield	" "
Peekskill	*J. Douglas Barry, M.D.	" "
Plattsburg	*Leo F. Schiff, M.D.	" "
Port Chester	W. J. Sheehan, M.D.	" "
Port Jervis	G. Otto Pobe, M.D.	" "
Poughkeepsie	W. H. Conger, M.D.	" "
Rensselaer	Dr. James C. Sharkey	" "
Rochester	*Arthur M. Johnson, M.D.	" "
Rockville Center	*A. D. Jacques, M.D.	" "
Rome	Lewis N. Eames, M.D.	" "
Saratoga Springs	Dr. Frederic J. Resseguie	" "
Schenectady	*J. B. Garlick, M.D.	Commissioner of Health
Syracuse	*Gregory D. Mahar, M.D.	" " "
Tonawanda	Dr. R. H. Wilcox	Health Officer
Troy	*James H. Flynn, M.D.	Health Commissioner
Utica	*Hugh H. Shaw, M.D.	Health Officer
Valley Stream	*John M. Quinn, M.D.	" "
Watertown	George B. Van Doren, M.D.	" "
Watervliet	Charles A. Birmingham, M.D.	Commissioner of Health
White Plains	**Edward H. Marsh, M.D.	Health Officer
Yonkers	*Louis V. Waldron, M.D.	Commissioner of Health
North Carolina		
Asheville	Dr. John W. Williams	Health Officer
Charlotte	Dr. G. L. Rea	" "
Concord	*Daniel Greenlee Caldwell, M.D.	County Health Officer
Durham	**Jesse H. Epperson, M.S.	Superintendent of Health
Elizabeth City	Dr. I. A. Ward	Health Officer
Fayetteville	M. T. Foster, M.D.	" "
Gastonia	*McG. Anders, M.D.	City Physician
Goldsboro	Dr. D. Fletcher Reeves	Director of Public Health
Greensboro	**C. C. Hudson, M.D.	Health Officer
High Point	Dr. W. J. McAnally	City Health Officer
Kinston	*Z. V. Moseley, M.D.	Health Officer

* Member, A.P.H.A. ** Fellow, A.P.H.A.

State and City	*Name of Health Officer*	*Official Title*
North Carolina (Cont.)		
New Bern	Dr. N. M. Gibbs	Health Officer
Raleigh	Dr. Carl V. Reynolds	Acting Health Officer
Rocky Mount	*Dr. J. Allen Whitaker	Superintendent of Health
Salisbury	**Charles Wallace Armstrong, M.D.	County Health Officer
Shelby	Dr. D. F. Moore	Health Officer
Statesville	Dr. R. S. McElwee	County Health Physician
Thomasville	*G. C. Gambrell, M.D.	Health Officer
Wilmington	*A. H. Elliot, M.D.	" "
Wilson	*W. H. Anderson, M.D.	" "
Winston-Salem	**R. L. Carlton, M.D.	" "
North Dakota		
Bismarck	Dr. Albert M. Fisher	Health Officer
Fargo	Dr. Harvey S. Skarshaug	" "
Grand Forks	Dr. J. M. Hofto	" "
Minot	Dr. J. L. Devine	" "
Ohio		
Akron	*Melville D. Ailes, M.D.	Director of Health
Alliance	Dr. Floyd R. Stamp	Health Officer
Ashland	Dr. Robert P. Bogniard	Director of Public Welfare
Ashtabula	Dr. James H. Park	City Health Officer
Barberton	H. A. Finefrock, M.D.	Health Commissioner
Bellaire	William E. Shepard, M.D.	Health Officer
Bucyrus	W. G. Carlisle, M.D.	Health Commissioner
Cambridge	Carl M. Osbe	" "
Campbell	James S. Mariner, M.D.	" "
Canton	F. M. Sayre, M.D.	" "
Chillicothe	*R. E. Bower, M.D.	Commissioner of Health
Cincinnati	*Owen C. Fisk, M.D.	Health Commissioner
Cleveland	**Harold J. Knapp, M.D.	" "
Cleveland Heights	*Robert Lockhart, M.D.	Director of Health
Columbus	*Nelson C. Dysart, M.D.	Health Commissioner
Coshocton	S. B. Kister, M.D.	" "
Cuyahoga Falls	**R. H. Markwith, M.D.	" "
Dayton	A. O. Peters, M.D.	Commissioner of Health
East Cleveland	George W. Stober, M.D.	Director of Health
East Liverpool	Dr. Roy C. Costello	Health Commissioner
Elyria	*G. E. French, M.D.	" "
Euclid	*Robert Lockhart, M.D.	Health Officer
Findlay	Marthy Laffey, R.N.	Health Commissioner
Fostoria	.....	" "
Fremont	E. L. Vermilya, M.D.	" "
Garfield Heights	*Robert Lockhart, M.D.	Health Officer
Hamilton	*C. J. Baldridge, M.D.	Health Commissioner
Ironton	H. S. Allen, M.D.	" "
Lakewood	Wallace J. Benner, M.D.	" "
Lancaster	Clifford E. Snider, M.D.	" "
Lima	James B. Poling, M.D.	" "
Lorain	*Valloyd Adair, M.D.	" "
Mansfield	*Millard C. Hanson, M.D.	" "
Marietta	Dr. F. S. McGee	" "
Marion	Dr. Kenneth D. Smith	Commissioner of Health
Martins Ferry	John Donovan	Health Commissioner
Massillon	Dr. Dwight L. Fisher	" "
Middletown	**G. D. Lummis, M.D.	Health Officer
Newark	W. H. Knauss, M.D.	Health Commissioner
New Philadelphia	**Joseph Blickensderfer, M.D.	" "
Niles	W. A. Werner, M.D.	" "
Norwood	Louis O. Saur, M.D.	" "
Painesville	*Clara Carter Wilder, R.N.	Health Officer
Parma	*Robert Lockhart, M.D.	" "
Piqua	L. G. Whitney	Health Commissioner
Portsmouth	O. D. Tatjo, M.D.	Health Officer
Salem	Dr. R. T. Holzbach	Health Commissioner
Sandusky	*F. M. Houghtaling, M.D.	" "
Shaker Heights	*Paul Marcus Spurney, M.D.	Health Officer
Springfield	*Oscar M. Craven, M.D.	Director of Public Health
Steubenville	*Julius A. Pizzoferrato	Health Commissioner
Struthers	Dr. Charles Scofield	Health Officer
Tiffin	*J. A. Goslin, M.D.	Commissioner of Health

* Member, A.P.H.A. ** Fellow, A.P.H.A.

State and City	*Name of Health Officer*	*Official Title*
Ohio (Cont.)		
Toledo	Dr. Basil B. Brim	Health Officer
Warren	M. T. Knappenberger, M.D.	Health Commissioner
Wooster	*William G. Rhoten, M.D.	" "
Xenia	Dr. A. D. De Haven	Health Officer
Youngstown	Dr. C. H. Beight	Health Commissioner
Zanesville	Dr. D. G. Candy	Supt. of Health & Sanitation
Oklahoma		
Ada	Dr. O. E. Welborn	City Health Officer
Ardmore	A. Y. Easterwood, M.D.	" " "
Bartlesville	Elizabeth Chamberlin, M.D.	Superintendent of Health
Chickasha	Dr. E. L. Dawson	" " "
Enid	R. C. Baker, M.D.	" " "
Lawton	*Fratis Duff	Health Officer
McAlester	.....	Superintendent of Health
Muskogee	Dr. I. T. Woodburn	City Physician
Oklahoma City	*W. H. Miles, M.D.	Health Director
Okmulgee	Raymond DeVoy	Sanitary Inspector
Ponca City	Mildred Headley, M.D.	Health Officer
Sapulpa	A. C. Frampton	Dairy & Health Inspector
Seminole	E. R. McAllister, M.D.	Health Officer
Shawnee	Dr. Leroy J. Neal	City Physician
Tulsa	Dr. J. Jeff Billington	Superintendent of Health
Wewoka	Dr. George Hunter	Health Officer
Oregon		
Astoria	*Nellie S. Vernon, M.D.	City Health Officer
Eugene	Dr. Donald C. Romig	" " "
Klamath Falls	Dr. G. S. Newsom	Health Officer
Medford	*Dr. C. I. Drummond	" "
Portland	*John G. Abele, M.D.	City Health Officer
Salem	*Vernon A. Douglas, M.D.	" " "
Pennsylvania		
Aliquippa	J. E. Tanner	Health Officer
Allentown	**J. Treichler Butz, M.D.	" "
Altoona	R. A. Herbert	Chief, Bureau of Health
Ambridge	Louis Herrmann	Health Officer
Arnold	Frank E. Morrison	Secretary, Board of Health
Beaver Falls	William Elmes, M.E.	Health Officer
Bellevue	James B. Arthur	" "
Berwick	C. E. Ross	" "
Bethlehem	Dr. F. J. Conahan	City Physician
Braddock	James E. Wills	Health Officer
Bradford	R. G. Vogel	" "
Bristol	John M. Wright	" "
Butler	J. Fred Leetch	" "
Cannonsburg	Frank Milligan	" "
Carbondale	Paul Nelson	Sanitary Officer
Carlisle	U. Grant Eppley	Health Officer
Carnegie	Joseph Lewis	" "
Chambersburg	Frank J. Croft	Health Officer
Charleroi	J. M. Hill	City Health Officer
Chester	Timothy McCarey	Health Officer
Clairton	F. F. Keller	" "
Coatesville	Charles V. Peace, V.M.D.	" "
Columbia	George M. Rodenhauser	" "
Connellsville	D. H. Minerd	" "
Conshohocken	Thomas S. White	Secretary, Board of Health
Coraopolis	Henry N. Holdren, Jr.	Health Officer
Dickson City	Joseph S. Kalwaytis	Secretary, Board of Health
Donora	Herman Lang	Health Officer
Dormont	Henry Chrystal	" "
DuBois	J. I. Brockbank, M.D.	" "
Dunmore	William Ferrese	" "
Duquesne	C. W. Goldstrohm	" "
Easton	Dr. J. S. Cohen	" "
Ellwood City	Louis Young	" "
Erie	**James R. Smith, M.D.	" "
Ferrell	Benjamin F. Davis	" "
Franklin	C. H. Brown, M.D.	" "

* Member, A.P.H.A. ** Fellow, A.P.H.A.

State and City	*Name of Health Officer*	*Official Title*
Pennsylvania (Cont.)		
Greensburg	Joseph B. Cherry	Health Officer
Hanover	Howard P. Knapper	Secretary, Board of Health
Harrisburg	**John M. J. Raunick, M.D.	Health Officer
Hazelton	W. H. Pfaff	" "
Homestead	M. D. Weis	" "
Jeanette	Charles E. Walter	" "
Johnstown	L. W. Jones, M.D.	" "
Kingston	J. F. Seward	" "
Lancaster	Benjamin F. Charles	" "
Latrobe	W. T. Osborne	" "
Lebanon	John D. Bogen, M.D.	" "
Lewistown	H. E. Fetteroff	" "
Mahoney City	Harry Martin	" "
McKeesport	Daniel F. Marsh	" "
McKees Rocks	Mary Dougherty	Secretary, Board of Health
Meadville	John Laley	Health Officer
Monesson	John Lermann	" "
Mount Carmel	Charles F. Cohoon	" "
Munhall	W. J. Caddy	Secretary, Board of Health
Nanticoke	J. H. Abbott	Health Officer
New Castle	*William L. Steen, M.D.	" "
New Kensington	John H. Evans	" "
Norristown	*R. Ronald Dettre	" "
North Braddock	George A. Shepard	" "
Oil City	*W. J. Lewis	" "
Old Forge	Primo Cesare	Chief of Police
Olyphant	Andrew Taras	Borough Health Officer
Philadelphia	*J. Norman Henry, M.D.	Director, Dept. of Public Health
Phoenixville	R. E. Deery	Health Officer
Pittsburgh	*R. P. Moyer, M.D.	Director, Dept. of Public Health
Pittston	Michael A. McHale	Health Officer
Plymouth	H. G. Templeton, M.D.	Secretary, Board of Health
Pottstown	A. John Andre	Health Officer
Pottsville	A. C. Huntzinger	" "
Reading	Ira J. Hain, M.D.	" "
Scranton	Dr. Arthur E. Davis	Director of Public Health
Shamokin	Fred Zeiser	Health Officer
Sharon	J. S. Hildebrand	Sanitary Officer
Shenandoah	Dr. Claude Davis	Health Officer
Steelton	E. G. Butler	" "
Sunbury	Carl P. Inkrote	" "
Swissvale	William H. Rushworth	" "
Tamaqua	Lamont Perrine	" "
Taylor	E. E. Edwards, M.D.	" "
Turtle Creek	Manuel Emmanuel	" "
Uniontown	W. C. Hall	" "
Vandergrift	J. D. Remaley	" "
Warren	Ralph N. Brown	" "
Washington	Thos. W. Henderson	" "
Waynesboro	Percy H. Snowberger	" "
West Chester	W. T. Garrett	Secretary, Board of Health
Wilkes Barre	**C. B. Crittenden, M.D.	Health Officer
Wilkinsburg	J. M. Snyder	" "
Williamsport	William J. Molenkopf	" "
York	J. Frank Small, M.D.	Director of Public Health
Rhode Island		
Bristol	Daniel E. Dwyer	Health Officer
Central Falls	Charles S. Doucet	Superintendent of Health
Cranston	*Daniel S. Latham, M.D.	" " "
East Providence	W. H. T. Hamill	Health Officer
Newport	*Edward V. Murphy, M.D.	Commissioner of Health
North Providence	Herbert A. Brown	Health Officer
Pawtucket	Dr. Albert L. Vandale	Superintendent of Health
Providence	*Michael J. Nestor, M.D.	City Health Officer
Warwick	*L. J. Smith, M.D.	Health Officer
West Warwick	D. S. Harrop, M.D.	" "
Westerly	Samuel C. Webster, M.D.	Superintendent of Health
Woonsocket	Dr. James P. O'Brien	Health Officer

* Member, A.P.H.A. ** Fellow, A.P.H.A.

State and City	*Name of Health Officer*	*Official Title*
South Carolina		
Anderson	**E. E. Epting, M.D.	Commissioner of Health
Charleston	**Leon Banov, M.D.	City & County Health Officer
Columbia	P. E. Payne	Health Officer
Florence	George D. Heath, M.D., Dr.P.H.	Health Commissioner
Greenville	**Irving S. Barksdale, M.D.	Commissioner of Health
Greenwood	Dr. Joseph E. Brodie	Health Officer
Rock Hill	Dr. R. D. Sumner	Medical Officer
Spartanburg	*J. M. Beeler, M.D.	Health Officer
Sumter	Dr. S. R. Kitchen	" "
South Dakota		
Aberdeen	Dr. J. F. Adams	City Health Officer
Huron	Dr. William H. Saxton	City Physician
Mitchell	Dr. E. M. Young	Health Officer
Rapid City	F. J. Austin, M.D.	" "
Sioux Falls	Dr. E. E. Gage	" "
Watertown	Dr. W. G. Magee	City Health Officer
Tennessee		
Bristol	*F. L. Moore, M.D.	Director, County Health Dept.
Chattanooga	Dr. J. W. L. Cooper	Health Officer
Jackson	Dr. Herman Hawkins	City Physician
Johnson City	*Dr. W. L. Poole	County Health Officer
Kingsport	*F. L. Moore, M.D.	Health Officer
Knoxville	*W. H. Enneis, M.D.	" "
Memphis	*L. M. Graves, M.D.	Superintendent of Health
Nashville	*John Overton, M.D.	City Health Officer
Texas		
Abilene	Scott Wingo Hollis, M.D.	City Health Officer
Amarillo	*B. M. Primer	Director, County Health Unit
Austin	*Eugene O. Chimene, M.D.	Director of Public Health
Beaumont	Dr. William W. Dunn	City Health Officer
Big Spring	H. M. Bennett, M.D.	Health Officer
Brownsville	Dr. Thurman A. Kinder, Jr.	City Health Officer
Brownwood	Dr. J. M. Horn	" " "
Cleburne	Dr. Joseph M. Stallicup	" " "
Corpus Christi	Dr. N. D. Carter	" " "
Corsicana	Dr. W. T. Shell, Jr.	" " "
Dallas	**J. W. Bass, M.D.	Director of Public Health
Del Rio	Dr. D. A. York	City Health Officer
Denison	W. A. Lee, M.D.	Health Officer
El Paso	*Dr. T. J. McCamant	Director, City-County Health Unit
Fort Worth	**A. H. Flickwir, M.D.	Health Officer
Galveston	*Walter Kleberg, M.D.	City Health Officer
Greenville	B. F. Arnold, M.D.	Health Officer
Harlingen	Dr. V. M. Bass	" "
Houston	*G. W. Larendon, M.D.	City Health Officer
Laredo	H. M. Austin	" " "
Lubbock	J. W. Rollo	Health Officer
Marshall	Dr. W. H. Bennett	City Health Officer
Palestine	John M. Colley, M.D.	Health Officer
Pampa	.....	" "
Paris	Dr. John A. Stephens	City Health Officer
Port Arthur	Dr. F. J. Beyt	" " "
San Angelo	Dr. B. T. Brown	" " "
San Antonio	**W. A. King, M.D.	Health Officer
San Benito	Dr. Neal D. Monger	City Health Officer
Sherman	Dr. C. D. Strather	Health Officer
Sweetwater	*Ernest W. Prothro, M.D.	Director of Public Health
Temple	Dr. Robert R. Curtis	City Health Officer
Texarkana	Dr. Charles A. Smith	" " "
Tyler	Albert Woldert	" " "
Waco	Dr. R. W. Crosthwait	" " "
Wichita Falls	.....	" " "
Utah		
Ogden	Dr. Walter E. Whalen	Director of Health Dept.
Provo	Dr. C. M. Smith	City Physician
Salt Lake City	Dr. Sol Kahn	Health Commissioner

* Member, A.P.H.A. ** Fellow, A.P.H.A.

State and City	Name of Health Officer	Official Title
Vermont		
Barre	Dr. Michael F. Cerasoli	Health Officer
Bennington	Joseph M. Ayres	" "
Burlington	Erald F. Foster, M.D.	" "
Rutland	*Clare M. Cole, M.D.	" "
Virginia		
Alexandria	W. L. Schafer, M.D.	Health Officer
Charlottesville	*C. Howe Eller, M.D., Dr.P.H.	" "
Danville	R. W. Garnett, M.D.	" "
Hopewell	L. A. Sims	City Engineer
Lynchburg	**Mosby G. Perrow, Ph.D.	Director of Public Welfare
Newport News	*G. Colbert Tyler, M.D.	" " " "
Norfolk	*J. C. Sleet, M.D.	Health Commissioner
Petersburg	*Mason Romaine, M.D.	Health Officer
Portsmouth	**Lonsdale J. Roper, M.D.	Director of Public Welfare
Richmond	**W. Brownley Foster, M.D.	" " " "
Roanoke	*Coleman B. Ransone, M.D.	Health Officer
Staunton	*J. F. Fulton, M.D.	" "
Suffolk	**William F. Wild, M.D.	Director of Health
Winchester	Dr. L. M. Allen	Health Officer
Washington		
Aberdeen	B. O. Swinehart, M.D.	City Health Officer
Bellingham	Dr. O. E. Beebe	" " "
Bremerton	D. H. Polk, M.D.	" " "
Everett	Dr. I. W. Parsons	" " "
Hoquiam	Dr. H. C. Watkins	" " "
Longview	Dr. J. S. McCarthy	Health Officer
Olympia	Dr. W. L. Bridgford	" "
Port Angeles	Will H. Taylor, M.D.	" "
Seattle	*F. M. Carroll, M.D.	Commissioner of Health
Spokane	**Ralph Hendricks, M.D.	Commissioner of Public Affairs
Tacoma	**S. M. Creswell, M.D.	Director of Health
Vancouver	Dr. Clyde B. Hutt	County Health Officer
Walla Walla	**Jerry E. Vanderpool, M.D.	County & City Health Officer
Wenatchee	*C. R. Fargher, M.D.	Health Officer
Yakima	**Lloyd Moffitt, M.D.	County & City Health Officer
West Virginia		
Bluefield	**David B. Lepper, M.D.	Director of Health
Charleston	*Hugh B. Robins, M.D.	Health Commissioner
Clarksburg	Dr. R. L. Osborne	City Health Officer
Fairmont	James A. Jamieson, M.D.	" " "
Huntington	Dr. Gilbert A. Ratcliff	Health Officer
Martinsburg	*C. A. Thomas, M.D.	" "
Morgantown	*R. C. Farrier, M.D.	County Health Officer
Moundsville	*W. G. C. Hill, M.D.	" " "
Parkersburg	**Arthur D. Knott, M.D., Dr.P.H.	City & County Health Officer
Wheeling	*Reece M. Pedicord, M.D.	City Health Commissioner
Wisconsin		
Appleton	Frank P. Dohearty, M.D.	City Health Officer
Ashland	Henry Wolfman	Health Commissioner
Beloit	Dr. R. S. Vivian	Health Officer
Cudahy	Dr. Bernard Krueger	" "
Eau Claire	*L. H. Flynn, M.D.	" "
Fond du Lac	Dr. M. O. Bowdry	" "
Green Bay	H. S. Atkinson, M.D.	Commissioner of Health
Janesville	Fred B. Welch, M.D.	City Health Officer
Kenosha	**Gustave Windesheim, M.D.	Health Officer
La Crosse	*A. M. Murphy	Acting Health Commissioner
Madison	*F. F. Bowman, M.D.	Health Officer
Manitowoc	Dr. George M. Hoffman	Health Commissioner
Marinette	*J. William Boren, M.D.	" "
Milwaukee	**John P. Koehler, M.D.	Commissioner of Health
Oshkosh	Dr. J. J. Kronzer	Health Officer
Racine	**I. F. Thompson, M.D.	" "
Sheboygan	*G. L. Hildebrand, M.D.	Commissioner of Public Health
Shorewood	*Jerome M. Jekel, M.D.	Health Commissioner

* Member, A.P.H.A. ** Fellow, A.P.H.A.

State and City	*Name of Health Officer*	*Official Title*
Wisconsin (Cont.)		
South Milwaukee	Dr. Robert D. Moray	Health Officer
Stevens Point	*F. R. Krembs, M.D.	Health Commissioner
Superior	Dr. P. G. McGill	" "
Two Rivers	A. P. Zlatnik, M.D.	Health Officer
Watertown	Dr. Felix H. Zimmermann	Health Commissioner
Waukesha	Frank M. Scheele, M.D.	Health Officer
Wausau	*L. F. Bugbee	" "
Wauwatosa	Dr. Roy T. Hansen	" "
West Allis	*Dr. Charles S. Stern	Health Commissioner
Wyoming		
Casper	Dr. J. C. Kamp	Health Officer
Cheyenne	Dr. H. R. Dillman	City Health Officer

* Member, A.P.H.A. ** Fellow A.P.H.A.

Full-time County Health Officers (540)

(As of February 1, 1936)

The United States Public Health Service list published in 1935 is the basis of the following. The variations represent later information received by the American Public Health Association through correspondence.

State and County	Name of Health Officer	Post Office	Official Title
Alabama			
Autuga	Dr. E. L. Trammell	Prattville	County health officer
Baldwin	Dr. S. A. Durick	Bay Minette	" " "
Barbour	E. M. Moore, M.D.	Clayton	" " "
Blount	*S. D. Sturkie, M.D.	Oneonta	" " "
Bullock	Dr. H. E. Barker	Union Springs	" " "
Calhoun	George A. Cryer, M.D.	Anniston	" " "
Chambers	Dr. W. J. Donald	Lafayette	" " "
Cherokee	*S. C. Tatum, M.D.	Center	" " "
Chilton	*Dr. J. M. Kimmey	Clanton	" " "
Cleburne	F. R. Wood, M.D.	Heflin	" " "
Colbert	Dr. G. W. Warrick	Tuscumbia	" " "
Conecuh	E. L. Kelly, M.D.	Evergreen	" " "
Covington	Dr. C. D. McLeod	Andalusia	" " "
Crenshaw	J. O. Foster, M.D.	Luverne	" " "
Cullman	*M. S. Whiteside, M.D.	Cullman	" " "
Dale	William L. Orr, M.D.	Ozark	" " "
Dallas	*L. Tennent Lee, M.D.	Selma	" " "
Elmore	Dr. C. S. Cotlin, Jr.	Wetumpka	" " "
Escambia	Dr. E. F. Goldsmith	Brewton	" " "
Etowah	*C. L. Murphree, M.D.	Gadsden	" " "
Franklin	N. P. Underwood, M.D.	Russellville	" " "
Houston	*F. G. Granger, M.D.	Dothan	" " "
Jackson	Dr. George E. Newton	Scottsboro	" " "
Jefferson	*J. D. Dowling, M.D.	Birmingham	" " "
Lamar	*Dr. W. J. B. Owings	Vernon	" " "
Lauderdale	*W. D. Hubbard, M.D.	Florence	" " "
Lawrence	R. E. Harper, M.D.	Moulton	" " "
Lee	H. C. McRee, M.D.	Opelika	" " "
Limestone	*Dr. W. A. Minsch	Athens	" " "
Lowndes	Dr. E. F. Leatherwood	Hayneville	" " "
Macon	Murray Smith, M.D.	Tuskegee	" " "
Madison	W. C. Hatchett, M.D.	Huntsville	" " "
Marengo	*E. T. Norman, M.D.	Linden	" " "
Marion	*W. T. Burkett, M.D.	Hamilton	" " "
Marshall	Dr. Lee Weathington	Guntersville	" " "
Mobile	*O. L. Chason, M.D.	Mobile	" " "
Monroe	*R. D. Neal, M.D.	Monroeville	" " "
Montgomery	*J. L. Bowman, M.D.	Montgomery	County & city health officer
Morgan	*L. R. Murphree, M.D.	Decatur	County health officer
Perry	J. R. Long, M.D.	Marion	" " "
Pickens	Dr. J. J. Croley	Carrollton	" " "
Pike	W. H. Abernethy, M.D.	Troy	" " "
Randolph	Dr. M. R. McWhorter	Wedowee	" " "
Russell	Dr. M. L. Shaddix	Phenix City	" " "
Shelby	Dr. H. C. Nickson	Columbiana	" " "
Sumter	Dr. S. J. Williams	Livingston	" " "
Talladega	J. H. Hill, M.D.	Talladega	" " "
Tallapoosa	C. C. Fargason, M.D.	Dadeville	" " "
Tuscaloosa	Dr. A. A. Kirk	Tuscaloosa	" " "
Walker	A. M. Waldrop, M.D.	Jasper	" " "
Washington	I. C. Sumner, M.D.	Chatom	" " "
Wilcox	E. L. McIntosh, M.D.	Camden	" " "
Winston	Dr. S. W. Shelton	Double Springs	" " "
Arizona			
Cochise	**R. B. Durfee, M.D.	Bisbee	County Supt. of P. H.
Gila	*Geoffrey Morris, M.D.	Globe	County health officer
Maricopa	**A. N. Crain, M.D.	Phoenix	" " "
Pima	*Lewis H. Howard, M.D.	Tucson	" " "

* Member, A.P.H.A. ** Fellow, A.P.H.A.

State and County	Name of Health Officer	Post Office	Official Title
Arkansas			
Ashley	*A. M. Gibbs, M.D.	Hamburg	County health officer
Clark	T. T. Ross, M.D.	Arkadelphia	" " "
Conway	A. B. Jemison, M.D.	Morrilton	Medical director
Crittenden	*B. M. Stevenson, M.D.	Marion	County health officer
Cross	J. L. Griffin, M.D.	Wynne	Medical director
Faulkner	Marcus T. Smith, M.D.	Conway	County health officer
Garland	J. F. Merritt, M.D.	Hot Springs	City health officer
Jackson	*M. B. Owens, M.D.	Newport	Medical director
Jefferson	W. H. Bruce, M.D.	Pine Bluff	Director of health dept.
Little River	J. W. Ringgold, M.D.	Ashdown	County health officer
Lonoke-Prairie-Arkansas	W. Myers Smith, M.D.	Lonoke	" " "
Mississippi	*Arthur M. Washburn, M.D.	Blytheville	" " "
Monroe	Dr. W. T. Scarlett	Clarendon	" " "
Ouachita	R. C. Kennerly, M.D.	Camden	" " "
Phillips	W. B. Bruce, M.D.	Helena	Medical director
Pope	A. B. Tate, M.D.	Russellville	" "
Pulaski	*J. A. Summers, M.D.	Little Rock	County health officer
Saline	Dr. D. W. Fulmer	Benton	" " "
Sebastian	*J. E. Johnson, M.D.	Fort Smith	" " "
Woodruff	J. F. Hays, M.D.	Augusta	" " "
Yell	*T. J. Pool, M.D.	Ola	Medical director
California			
Alameda	**I. O. Church, M.D.	Oakland	County health officer
Contra Costa	*W. A. Powell, M.D.	Martinez	" " "
Fresno	*William F. Stein, M.D.	Fresno	" " "
Imperial	**Warren F. Fox, M.D.	El Centro	" " "
Kern	*Dr. Joe Smith	Bakersfield	" " "
Los Angeles	**J. L. Pomeroy, M.D.	Los Angeles	" " "
Madera	*Lee A. Stone, M.D.	Madera	" " "
Monterey	**Roy M. Fortier, M.D.	Salinas	" " "
Orange	*K. H. Sutherland, M.D.	Santa Ana	" " "
Riverside	*W. A. Jones, M.D.	Riverside	Commissioner of health
San Bernardino	*E. F. Godfrey, M.D.	San Bernardino	County health officer
San Joaquin	**John J. Sippy, M.D.	Stockton	" " "
San Diego	*Alex. M. Lesem, M.D.	San Diego	" " "
San Luis Obispo	**Allen F. Gillihan, M.D.	San Luis Obispo	" " "
San Mateo	*Charles C. Gans, M.D.	San Mateo	" " "
Santa Barbara	**R. C. Main, M.D.	Santa Barbara	" " "
Santa Clara	**C. M. Burchfield, M.D.	San Jose	" " "
Stanislaus	*E. F. Reamer, M.D.	Modesto	" " "
Ventura	Dr. A. A. Maulhardt	Ventura	" " "
Delaware			
Kent	*E. F. Smith, M.D.	Dover	County health officer
New Castle	*J. R. Downes, M.D.	Newark	" " "
Sussex	*Dr. F. I. Hudson	Georgetown	" " "
Florida			
Escambia	**W. H. Pickett, M.D.	Pensacola	County health officer
Jackson	Dr. Paul G. Shell	Mariana	" " "
Leon	L. J. Graves, M.D.	Tallahassee	" " "
Georgia			
Baldwin	O. F. Moran, M.D.	Milledgeville	Commissioner of health
Bartow	*A. C. Shamblin, M.D.	Cartersville	County health officer
Bibb	*J. D. Applewhite, M.D.	Macon	" " "
Brooks	.....	Quitman	" " "
Chatham	**Victor H. Bassett, M.D.	Savannah	" " "
Clarke	*W. W. Brown, M.D.	Athens	Health commissioner
Cobb	J. E. Lester, M.D.	Marietta	Commissioner of health
Colquitt	T. H. Chestnut, M.D.	Moultrie	County health officer
Decatur	M. A. Fort, M.D.	Bainbridge	" " "
DeKalb	J. R. Evans, M.D.	Decatur	" " "
Dougherty	Hugo Robinson, M.D.	Albany	Health commissioner
Floyd	B. V. Elmore, M.D.	Rome	Commissioner of health
Fulton	W. N. Gilbert, M.D.	Atlanta	County health officer
Glynn, McIntosh, Camden	**M. E. Winchester, M.D.	Brunswick	Commissioner of health
Grady	Dr. Howard L. Rankin	Cairo	County health officer
Hall	C. J. Wellborn, M.D.	Gainesville	" " "

* Member, A.P.H.A. ** Fellow, A.P.H.A.

State and County	Name of Health Officer	Post Office	Official Title
Georgia (Cont.)			
Jefferson	L. R. Bryson, M.D.	Louisville	County health officer
Jenkins	Hugh B. Senn, M.D.	Millen	" " "
Laurens	O. H. Cheek, M.D.	Dublin	Commissioner of health
Lowndes	G. T. Crozier, M.D.	Valdosta	County health officer
Mitchell	C. O. Rainey, M.D.	Camilla	Commissioner of health
Richmond	**H. Grady Callison, M.D.	Augusta	County health officer
Spalding	W. C. Humphries, M.D.	Griffin	" " "
Sumter	Dr. A. J. Davis	Americus	Commissioner of health
Thomas	James R. Dykes, M.D.	Thomasville	" " "
Troup	S. C. Rutland, M.D.	La Grange	" " "
Ware	*George E. Atwood, M.D.	Waycross	County health officer
Washington	*O. L. Rogers, M.D.	Sandersville	" " "
District Health Unit No. 1 Catoosa Walker	*C. W. Folsom, M.D.	Lafayette	Commissioner of health
Indiana			
Lake	*William D. Weis, M.D.	Crown Point	County health officer
Iowa			
Woodbury	*W. S. Petty, M.D.	Sioux City	Field Agent, U.S.P.H.S.
Kansas			
Lyon	C. Herbert Munger, M.D.	Emporia	County health officer
Sedgwick	*J. C. Montgomery, M.D.	Wichita	" " "
Shawnee	*F. E. McCord, M.D.	Topeka	" " "
Kentucky			
Adair	N. A. Mercer, M.D.	Columbia	County health officer
Allen	C. W. Holland, M.D.	Scottsville	" " "
Anderson	*S. R. Boggess, M.D.	Lawrenceburg	" " "
Ballard	Dr. Charles B. Billington	Wickliffe	" " "
Barren	.....	Glasgow	" " "
Bath	J. S. Goodpaster, M.D.	Owingsville	" " "
Bell	Dr. Adam Stacy, Jr.	Pineville	" " "
Boyd	Dr. Lee A. Dare	Ashland	Director of health
Breathitt	Dr. Earl E. Gambill	Jackson	County health officer
Butler	C. C. Threlkel, M.D.	Morgantown	" " "
Caldwell	Dr. B. K. Amos	Princeton	" " "
Calloway	James A. Outland, M.D.	Murray	" " "
Carlisle	J. F. Harrell, M.D.	Bardwell	Director, Co. H.D.
Carter	Dr. Don E. Wilder	Grayson	" " "
Casey	*J. W. Scudder, M.D.	Liberty	County health officer
Clay	Dr. L. H. Wagers	Manchester	" " "
Clinton	Dr. W. G. Morgan	Albany	" " "
Edmonson	Dr. H. H. Bishop	Brownsville	" " "
Elliott	Dr. R. E. Wehr	Sandy Hook	" " "
Estill	Dr. R. R. Snowden	Irvine	Director, Co. H.D.
Fayette	Dr. Charles O. Cawood	Lexington	County health officer
Fleming	Dr. C. W. Christine	Flemingsburg	" " "
Floyd	Marvin Ransdell, M.D.	Prestonburg	Director, Co. H.D.
Fulton	Hugh E. Prather, M.D.	Hickman	Director of health
Gallatin	Dr. J. W. Miller	Warsaw	County health officer
Grant	N. H. Ellis, M.D.	Williamstown	" " "
Grayson	Dr. J. G. Samuels, Jr.	Leitchfield	" " "
Green	Dr. J. M. Dishman	Greensburg	" " "
Greenup	*Dr. C. M. Gambill	Greenup	" " "
Hart	Charles P. Shields, M.D.	Munfordsville	" " "
Henderson	Dr. J. Leland Tanner	Henderson	" " "
Hickman	Charles Hunt, M.D.	Clinton	Director, Co. H.D.
Hopkins	*C. R. Morton, M.D.	Madisonville	" " "
Jefferson	*J. D. Trawick, M.D.	Louisville	County health officer
Kenton	H. C. White, M.D.	Covington	" " "
Knott	*J. W. Duke, M.D.	Hindman	" " "
Knox	*W. V. Bradshaw, Jr., M.D.	Barbourville	" " "
Laurel	*G. S. Brock, M.D.	London	" " "
Lawrence	W. C. Gose, M.D.	Louisa	Director, Co. H.D.
Lee	E. M. Brown, M.D.	Beattyville	County health officer
Leslie	Dr. D. D. Turner	Hyden	" " "

* Member, A.P.H.A. ** Fellow A.P.H.A.

State and County	Name of Health Officer	Post Office	Official Title
Kentucky (Cont.)			
Letcher	Dr. H. A. Shields	Whitesburg	County health officer
Lincoln	Dr. K. T. Johnstone	Stanford	" " "
Lyon	Dr. N. M. Atkins	Eddyville	" " "
McCracken	.....	Paducah	" " "
McCreary	Dr. Wallace M. Chapman	Whitley City	" " "
McLean	*G. L. Thompson, M.D.	Calhoun	Director, Co. H.D.
Madison	Dr. H. G. Wells	Richmond	County health officer
Magaffin	Dr. H. K. Bailey	Salyersville	Director, Co. H.D.
Marshall	S. L. Henson, M.D.	Benton	County health officer
Martin	William N. Keith, M.D.	Inez	Director, Co. H.D.
Mason	*O. M. Goodloe, M.D.	Maysville	County health officer
Meade	O. R. Lynch, M.D.	Brandenburg	" " "
Menifee	E. T. Riley, M.D.	Frenchburg	Director, Co. H.D.
Metcalfe	Dr. H. T. Carter	Edmonton	County health officer
Monroe	Dr. A. S. Yates	Tompkinsville	" " "
Muhlenberg	Roy M. Orsburn, M.D.	Greenville	" " "
Nicholas	Dr. E. W. Atherton	Carlisle	" " "
Ohio	A. D. Park, M.D.	Hartford	" " "
Owsley	Dr. J. R. Aker	Booneville	Director, Co. H.D.
Perry	*D. D. Carr, M.D.	Hazard	County health officer
Pike	R. E. Teague, M.D.	Pikeville	" " "
Powell	Dr. S. T. Scrivner	Stanton	" " "
Pulaski	*D. A. Reekie, M.D.	Somerset	" " "
Rockcastle	Walker Owens, M.D.	Mt. Vernon	" " "
Rowan	*T. A. E. Evans, M.D.	Morehead	" " "
Scott	Dr. R. J. Griffin	Georgetown	Director of health
Spencer	Dr. M. W. Caskey	Taylorsville	County health officer
Todd	L. A. Crosby, M.D.	Elkton	" " "
Trigg	Dr. E. W. Sigler	Cadiz	" " "
Trimble	*J. J. Gerkins, M.D.	Bedford	" " "
Union	Dr. A. Y. Covington	Morganfield	" " "
Warren	*G. M. Wells, M.D.	Bowling Green	" " "
Wayne	Mack Roberts, M.D.	Monticello	" " "
Webster	C. M. Smith, M.D.	Dixon	Director, Co. H.D.
Wolfe	Dr. J. L. Cox	Campton	" " "
Louisiana			
Acadia	*J. D. Hunter, M.D.	Crowley	County health officer
Assumption	**P. M. Payne, M.D.	Napoleonville	Director of health
Avoyelles	Dr. L. W. Holloman	Marksville	Acting director of health
Caddo	*W. J. Sandidge, M.D.	Shreveport	Director of health
Caldwell	Thomas Burke, M.D.	Columbia	" " "
Catahoula	L. C. Spencer, M.D.	Harrisburg	" " "
Claiborne	*H. R. Marlatt, M.D.	Homer	County health officer
Concordia	John Schreiber, M.D.	Vidalia	" " "
De Soto	*R. A. Tharp, M.D.	Mansfield	Director of health
East Carroll	Dr. F. V. Boyd	Lake Providence	County health officer
Franklin	R. E. Applewhite, M.D.	Winnsboro	Director of health
Iberia	B. L. Stinson, M.D.	New Iberia	" " "
Iberville	*J. C. Eby, M.D.	Plaquemine	County health officer
Lafayette	A. J. Comeaux, M.D.	Lafayette	Director of health
Lafourche	H. S. Smith, M.D.	Thibodaux	" " "
La Salle	E. L. Miller, M.D.	Jena	" " "
Lincoln	R. H. Allen, M.D.	Ruston	County health officer
Madison	E. S. Freeman, M.D.	Tallulah	" " "
Morehouse	N. P. Liles, M.D.	Bastrop	Director of health
Natchitoches	*W. W. Knipmeyer, M.D.	Natchitoches	" " "
Ouchita	G. D. Williams, M.D.	Monroe	" " "
Pointe Coupee	*F. F. Rougon, M.D.	New Roads	County health officer
Rapides	*B. J. Aymond, M.D.	Alexandria	" " "
Red River	*Bernard Hochfelder, M.D.	Coushatta	" " "
Richland	R. O. C. Green, M.D.	Rayville	Co. director of health
St. Landry	Dr. L. A. Masterson	Opelousas	County health officer
St. Martin	P. H. Fleming, M.D.	St. Martinsville	Co. director of health
St. Mary	Dr. W. W. Poimboeuf	Franklin	Director of health
Tensas	*J. G. Norris, M.D.	St. Joseph	County health officer
Terrebonne	M. F. Houston, M.D.	Houma	" " "
Washington	F. A. Williams, M.D.	Franklinton	Director of health
Webster	*W. C. Summer, M.D.	Minden	" " "
West Carroll	Dr. F. S. Williams	Oak Grove	" " "

* Member, A.P.H.A. ** Fellow A.P.H.A.

State and County	Name of Health Officer	Post Office	Official Title
Maine			
Cooperative Health Union	**B. L. Arms, M.D.	Farmington	County health officer
Motbov Union	H. L. Jackson, M.D.	Old Town	Health officer
Maryland			
Allegany	J. P. Franklin, M.D.	Cumberland	County health officer
Anne Arundel	*John H. Janney, Jr., M.D.	Annapolis	" " "
Baltimore	*J. S. Bowen, M.D.	Towson	" " "
Calvert	*I. N. King, M.D.	Prince Frederick	" " "
Caroline	*Louis S. Welty, M.D.	Denton	" " "
Carroll	W. C. Stone, M.D.	Westminster	" " "
Cecil	*C. A. Kane, M.D.	Elkton	" " "
Charles	.....	La Plata	" " "
Dorchester	**E. A. Jones, M.D.	Cambridge	" " "
Frederick	*E. C. Kefauver, M.D.	Frederick	" " "
Garrett	*Eugene C. Peck, M.D.	Oakland	" " "
Harford	*T. A. Callahan, M.D.	Bel Air	" " "
Howard	*William J. French, M.D.	Ellicott City	" " "
Kent	**R. G. Beachley, M.D., D.P.H.	Chestertown	" " "
Montgomery	**V. L. Ellicott, M.D., Dr.P.H.	Rockville	" " "
Prince Georges	*A. B. Hooton, M.D.	Upper Marlboro	" " "
Queen Annes	James A. McCallum, M.D.	Centreville	" " "
St. Marys	Dr. D. St. Clair Campbell	Leonardtown	" " "
Somerset	Robert H. Johnson, M.D.	Princess Anne	" " "
Talbot	*George C. Halley, M.D.	Easton	" " "
Washington	W. Ross Cameron, M.D.	Hagerstown	" " "
Wicomico	*Seth H. Hurdle, M.D.	Salisbury	" " "
Worcester	*Bradford Massey, M.D.	Pocomoke	" " "
Massachusetts			
Barnstable	*A. P. Goff, M.D.	Hyannis	County health officer
Nashoba District	*James O. Wails, M.D.	Ayer	Medical director
Southern Berkshire District	**H. W. Stevens, M.D.	Great Barrington	Medical director
Michigan			
Allegan	Dr. S. E. Beckett	Allegan	County health officer
Barry	*Robert B. Harkness, M.D.	Hastings	" " "
Branch	*Dr. F. S. Leeder	Coldwater	" " "
Calhoun	*Dr. M. R. Kinde	Marshall	" " "
Eaton	*J. W. Davis, M.D.	Charlotte	" " "
Genesee	Dr. T. E. Gibson	Flint	" " "
Hillsdale	*Dr. E. G. McGavran	Hillsdale	" " "
Isabella	*Dr. F. R. Town	Mount Pleasant	" " "
Kent	*J. D. Brook, M.D.	Grand Rapids	" " "
Midland	**Dr. David Littlejohn	Midland	" " "
Oakland	**John D. Monroe, M.D.	Pontiac	" " "
Ottawa	*Ralph Ten Have, M.D.	Grand Haven	" " "
Saginaw	**V. K. Volk, M.D.	Saginaw	" " "
Van Buren	*Theodore R. Meyer, M.D.	Paw Paw	" " "
Wexford	*S. C. Moore, M.D.	Cadillac	" " "
District No. 1	*T. R. Laughbaum, M.D.	Lake City	" " "
District Health Unit	*Guy R. Post, M.D.	White Cloud	" " "
District Health Unit	Dr. Gladys Kleinschmidt	West Branch	" " "
District Health Unit	*Carlteton Dean, M.D.	Charlevoix	" " "
District Health Unit	*Dr. Gordon B. Moffat	Rogers City	" " "
District Health Unit	**Clarence D. Hart, M.D.	Newberry	" " "
District Health Unit	**Edward V. Thiehoff, M.D.	Gladwin	" " "
Minnesota			
St. Louis	*C. A. Scherer, M.D.	Duluth	County health officer
Mississippi			
Adams	*A. R. Perry, M.D.	Natchez	County health officer
Bolivar	*R. D. Dedwylder, M.D.	Cleveland	Director of health
Calhoun	F. L. McGahey	Calhoun City	County health officer
Coahoma	*N. C. Knight, M.D.	Clarksdale	" " "
Copiah	*J. C. McGuire, M.D.	Hazlehurst	" " "
Forrest	*B. D. Blackwelder, M.D.	Hattiesburg	" " "
Hancock	C. M. Shipp, M.D.	Bay St. Louis	Director of health
Harrison	D. J. Williams, M.D.	Gulfport	County health officer

* Member, A.P.H.A. ** Fellow A.P.H.A.

State and County	Name of Health Officer	Post Office	Official Title
Mississippi (Cont.)			
Hinds	W. E. Noblin, M.D.	Jackson	County health officer
Holmes	Dr. C. C. Smith	Lexington	" " "
Humphreys	*J. W. Barkley, M.D.	Belzoni	Director of health
Jackson	*R. G. Lander, M.D.	Pascagoula	" " "
Lamar	J. N. Mason, M.D.	Purvis	County health officer
Lauderdale	*D. V. Galloway, M.D.	Meridian	" " "
Leake	Dr. W. S. Martin	Carthage	" " "
Lee	*W. H. Cleveland, M.D.	Tupelo	" " "
Leflore	*L. A. Barnett, M.D.	Greenwood	Director of health
Lincoln	*W. R. May, M.D.	Brookhaven	County health officer
Monroe	*C. H. Love, M.D.	Aberdeen	" " "
Newton	Dr. William A. McMahen	Union	" " "
Panola	Albert P. Alexander	Como	" " "
Pearl River	G. E. Godman, M.D.	Poplarville	Director of health
Pike	**T. Paul Haney, Jr., M.D.	McComb	County health officer
Sharkey	A. K. Barrier, M.D.	Rolling Fork	" " "
Sunflower	*H. B. Cottrell, M.D.	Indianola	" " "
Tallahatchie	Dr. G. T. Biles	Sumner	" " "
Union	*Irvin B. Trapp, M.D.	New Albany	" " "
Warren	*F. Michael Smith, M.D.	Vicksburg	Director
Washington	*J. W. Shackelford, M.D.	Greenville	County health officer
Winston	Dr. H. B. Watkins	Noxapater	" " "
Yazoo	*Hugh L. McCalip, M.D.	Yazoo City	" " "
Missouri			
Buchanan	*W. S. Hull, M.D.	St. Joseph	County health officer
Dunklin	*Wheeler Davis, M.D.	Kennet	" " "
Greene	*R. L. Russell, M.D.	Springfield	" " "
Jackson	*J. T. Brennan, M.D.	Independence	" " "
Marion	*Eugene M. Lucke, M.D.	Hannibal	Field agent
Miller	*L. M. Garner, M.D.	Tuscumbia	County health officer
New Madrid	William N. O'Bannon, M.D.	New Madrid	" " "
Pemiscot	Fred L. Ogilvie, M.D.	Caruthersville	" " "
St. Louis	*L. C. Obrock, M.D.	Clayton	" " "
Montana			
Cascade	**F. L. Watkins, M.D.	Great Falls	County health officer
Gallatin	*A. D. Brewer, M.D.	Bozeman	" " "
Missoula	**F. D. Pease, M.D.	Missoula	Health officer
New Mexico			
District I	*E. F. McIntyre	Santa Fe	District health officer
District II	*E. B. Beaver, M.D.	Gallup	" " "
District III	**J. R. Scott, M.D.	Albuquerque	" " "
District IV	**C. W. Gerber, M.D.	Las Cruces	" " "
District V	**W. W. Johnston, M.D.	Las Vegas	" " "
District VI	*O. E. Puckett, M.D.	Carlsbad	" " "
District VII	F. W. Parker, M.D.	Silver City	" " "
District VIII	*J. O. Long, M.D.	Los Lunas	" " "
District IX	*Frank C. Diver, M.D.	Raton	" " "
District X	*Leonard A. Dewey	Clovis	" " "
New York			
Cattaraugus	**Henry R. O'Brien, M.D.	Olean	Commissioner of health
Columbia	*Louis Van Hoesen, M.D.	Hudson	" " "
Cortland	**Merle R. French, M.D.	Cortland	" " "
Suffolk	*Arthur T. Davis, M.D.	Riverhead	" " "
Westchester	**Matthias Nicoll, Jr., M.D.	White Plains	" " "
District			
Fulton-Montgomery	*J. E. Perkins, M.D., D.P.H.	Amsterdam	Asst. dist. state health off.
Tompkins	**V. A. Van Volkenburgh	Ithaca	" " " " "
North Carolina			
Avery-Watauga-Yancey	Dr. C. H. White	Burnsville	County health officer
Beaufort	D. E. Ford, M.D.	Washington	" " "
Bladen	R. S. Cromartie, M.D.	Elizabethtown	" " "
Buncombe	*H. L. Sumner, M.D.	Asheville	" " "
Cabarrus	*Daniel G. Caldwell, M.D.	Concord	" " "
Columbus	Floyd Johnson, M.D.	Whiteville	" " "

* Member, A.P.H.A. ** Fellow A.P.H.A.

State and County	Name of Health Officer	Post Office	Official Title
North Carolina (Cont.)			
Cumberland	M. T. Foster, M.D.	Fayetteville	County health officer
Davidson	*G. C. Gambrell, M.D.	Lexington	" " "
Durham	**Jesse H. Epperson	Durham	" " "
Edgecombe	*A. D. Gregg, M.D.	Tarboro	" " "
Forsyth	*J. R Hege, M.D.	Winston-Salem	" " "
Franklin	Dr. R. L. Yarborough	Louisburg	" " "
Gaston	*R. E. Rhyne, M.D.	Gastonia	" " "
Granville	*J. A. Morris, M.D.	Oxford	" " "
Guilford	R. M. Buie, M.D.	Greensboro	" " "
Halifax	*R. S. McGeachy, M.D.	Weldon	" " "
Haywood-Jackson-Swain	Dr. C. N. Sisk	Waynesville	" " "
Hyde	Dr. S. V. Lewis	Ocracoke	" " "
Lenoir	*Z. V. Moseley, M.D.	Kinston	" " "
Mecklenburg	*E. H. Hand, M.D.	Charlotte	" " "
Moore	*John Symington, M.D.	Carthage	" " "
Nash	*T. O. Coppedge, M.D.	Nashville	" " "
New Hanover	*A. H. Elliot, M.D.	Wilmington	" " "
Northampton	M. H. Seawell, M.D.	Jackson	" " "
Orange-Person Dist.	Dr. M. H. Rourk	Hillsboro	" " "
Pitt	Dr. N. T. Ennett	Greenville	" " "
Randolph	*G. H. Sumner, M.D.	Asheboro	" " "
Richmond	*B. B. Dalton, M.D.	Rockingham	" " "
Roberson	*E. R. Hardin, M.D.	Lumberton	" " "
Rowan	**Charles W. Armstrong, M.D.	Salisbury	" " "
Rutherford	*R. M. Bardin, M.D.	Rutherfordton	" " "
Sampson	Dr. W. P. Starling	Clinton	" " "
Surry	Dr. R. J. Sykes	Mount Airy	" " "
Vance	*Z. P. Mitchell, M.D.	Henderson	" " "
Wake	*A. C. Bulla, M.D.	Raleigh	Health officer
Wayne	G. F. Reeves, M.D.	Goldsboro	County health officer
Wilkes	A. J. Eller, M.D.	Wilkesboro	" " "
Wilson	*W. H. Anderson, M.D.	Wilson	" " "
Ohio			
Butler	*C. J. Baldridge, M.D.	Hamilton	Commissioner of health
Clinton	W. K. Ruble, M.D.	Wilmington	Dist. health commissioner
Coshocton	Dr. W. A. McMichael	Coshocton	" " "
Crawford	*G. T. Wasson, M.D.	Bucyrus	" " "
Cuyahoga	*Robert Lockhart, M.D.	Cleveland	" " "
Darke	*W. D. Bishop, M.D.	Greenville	County health officer
Delaware	B. B. Barber, M.D.	Delaware	" " "
Erie	*F. M. Houghtaling, M.D.	Sandusky	Health commissioner
Fayette	*James F. Wilson, M.D.	Washington Court House	Dist. health commissioner
Hamilton	*E. H. Schoenling, M.D.	Cincinnati	County health officer
Hancock	S. F. Whisler, M.D.	Findlay	Dist. health commissioner
Hocking	W. B. Lacock, M.D.	Logan	" " "
Huron	B. C. Pilkey, M.D.	Norwalk	" " "
Jefferson	J. P. Young, M.D.	Steubenville	" " "
Lorain	**F. R. Dew, M.D.	Oberlin	County health officer
Lucas	Dr. C. W. Mahoney	Toledo	" " "
Mahoning	*G. Y. Davis, M.D.	Youngstown	Dist. health commissioner
Marion	*N. Sifritt, M.D.	Marion	County health officer
Medina	*John L. Jones, M.D.	Medina	" " "
Meigs	W. S. Ellis, M.D.	Pomeroy	Dist. health commissioner
Mercer	F. E. Ayers, M.D.	Celina	" " "
Miami	*E. R. Hiatt, M.D.	Troy	" " "
Montgomery	*H. H. Pansing, M.D.	Dayton	" " "
Perry	F. J. Crosbie, M.D.	New Lexington	" " "
Preble	J. I. Nisbet, M.D.	Eaton	County health officer
Richland	*M. C. Hanson, M.D.	Mansfield	" " "
Ross	*R. E. Bower, M.D.	Chillicothe	Dist. health commissioner
Scioto	*G. W. Fishbaugh, M.D.	Portsmouth	County health officer
Seneca	J. J. Heaton, M.D.	Tiffin	Dist. health commissioner
Shelby	*A. B. Lippert, M.D.	Sidney	" " "
Stark	Floyd Stamp, M.D.	Canton	" " "
Summit	**R. H. Markwith, M.D.	Akron	" " "
Trumbull	L. A. Connell, M.D.	Warren	" " "
Tuscarawas	**Joseph Blickensderfer, M.D.	New Philadelphia	" " "
Washington	A. G. Sturgiss, M.D.	Marietta	" " "

* Member, A.P.H.A. ** Fellow A.P.H.A.

State and County	Name of Health Officer	Post Office	Official Title
Ohio (Cont.)			
Wayne	*W. G. Rhoten, M.D.	Wooster	City & county health officer
Wood	*H. J. Powell, M.D.	Bowling Green	Dist. health commissioner
Oregon			
Jackson	*A. N. Johnson, M.D.	Medford	County health officer
Klamath	G. S. Newson, M.D.	Klamath Falls	" " "
Lane	R. C. Romig, M.D.	Eugene	" " "
Marion	*Vernon Douglas, M.D.	Salem	" " "
Multnomah	Dr. H. R. Cliff	Portland	" " "
South Carolina			
Aiken	J. T. Hair, M.D.	Aiken	County health officer
Anderson	**E. E. Epting, M.D.	Anderson	" " "
Beaufort	Dr. William A. Carrigan	Beaufort	" " "
Berkeley	*W. K. Fishburne, M.D.	Moncks Corner	" " "
Charleston	**Leon Banov, M.D.	Charleston	" " "
Cherokee	*E. P. White, M.D.	Gaffney	" " "
Darlington	*G. B. Edwards, M.D.	Darlington	" " "
Dillon-Marion	G. E. McDaniel, M.D.	Dillon	" " "
Dorchester	Dr. B. M. Montgomery	St. George	" " "
Fairfield	J. L. Bryson, M.D.	Winnsboro	" " "
Florence	J. R. Claussen, M.D.	Florence	" " "
Georgetown	S. S. Simons, M.D.	Georgetown	" " "
Greeneville	*Baylis Earle, M.D.	Greenville	" " "
Greenwood	J. E. Brodie, M.D.	Greenwood	" " "
Horry	.....	Conway	" " "
Kershaw	A. W. Humphries, M.D.	Camden	" " "
Newberry	H. B. Senn, M.D.	Newberry	" " "
Oconee	*B. F. Sloan, M.D.	Walhalla	" " "
Orangeburg	G. C. Bolin, M.D.	Orangeburg	" " "
Pickens	*W. B. Furman, M.D.	Pickens	" " "
Richland	*R. W. Ball, M.D.	Columbia	" " "
Spartanburg	*J. Moss Beeler, M.D.	Spartanburg	" " "
Tennessee			
Blount	O. F. Agee, M.D.	Maryville	County health officer
Bradley	*C. C. Sanford, M.D.	Cleveland	" " "
Davidson	*J. J. Lentz, M.D.	Nashville	" " "
Gibson	*F. L. Roberts, M.D.	Trenton	" " "
Giles	*Dr. J. U. Speer	Pulaski	" " "
Greene	*R. S. Cowles, M.D.	Greeneville	" " "
Grundy	*V. B. Bowden, M.D.	Pelham	" " "
Hamilton	J. C. Eldridge, M.D.	Chattanooga	" " "
Hardeman	*R. L. Cobb, M.D.	Bolivar	" " "
Hardin	*J. W. Erwin, M.D.	Savannah	" " "
Humphreys	Dr. S. D. Aycock	Waverly	" " "
Knox	*A. G. Hufstedler, M.D.	Knoxville	" " "
Lake	James P. Moon, M.D.	Tiptonville	Director of health
Lauderdale	*R. B. Griffin, M.D.	Ripley	County health officer
Lincoln	*M. F. Brown, M.D	Fayetteville	" " "
Maury	*H. C. Busby, M.D.	Columbia	" " "
Monroe	**David M. Cowgill, M.D.	Madisonville	" " "
Montgomery	F. J. Malone, M.D.	Clarksville	" " "
Obion	*W. B. Harrison, M.D.	Union City	" " "
Roane	*J. C. Fly, M.D.	Kingston	" " "
Rutherford	*J. B. Black, M.D.	Murfreesboro	" " "
Sevier	Dr. R. C. Kash	Sevierville	" " "
Shelby	*W. P. Moore, M.D.	Memphis	" " "
Sullivan	*F. L. Moore, M.D.	Blountville	" " "
Sumner	*H. M. Kelso, M.D.	Gallatin	Health officer
Tipton	A. J. Butler, M.D.	Covington	County health officer
Washington	*Dr. W. L. Poole	Jonesboro	Health officer
Weakley	*M. D. Ingram, M.D.	Dresden	County health officer
Williamson	*R. K. Galloway, M.D.	Franklin	" " "
Wilson	*B. W. Patton, M.D.	Lebanon	" " "
Districts			
Anderson-Campbell	*R. B. Howard, M.D.	Clinton	" " "
Carter-Unicoi	.....	Elizabethton	" " "
Claiborne-Grainger	*Alex B. Shipley, M.D.	Tazewell	" " "
Bledsoe-Sequatchie	*H. M. Roberson, M.D.	Pikeville	" " "
Jackson-Fentress	*F. B. Clark, M.D.	Gainesboro	" " "

* Member, A.P.H.A. ** Fellow A.P.H.A.

State and County	Name of Health Officer	Post Office	Official Title
Texas			
Cameron	W. A. Spivey, M.D.	San Benito	County health officer
Dallas	*H. E. Duncan, M.D.	Dallas	" " "
El Paso	*T. J. McCamant, M.D.	El Paso	" " "
Gregg	*T. B. Wilson, M.D.	Longview	" " "
Hidalgo	*D. R. Handley, M.D.	Edinburg	" " "
Nolan	*E. W. Prothro, M.D.	Sweetwater	" " "
Potter	*B. M. Primer, M.D., M.P.H.	Amarillo	" " "
Tarrant	Burke Brewster, M.D.	Fort Worth	" " "
Utah			
Davis	Sumner Gleason, M.D.	Kaysville	County health officer
Virginia			
Albemarle	*C. Howe Eller, M.D.	Charlottesville	County health officer
Arlington	P. M. Chichester, M.D.	Clarendon	" " "
Augusta	*H. M. Wallace, M.D.	Staunton	" " "
Brunswick-Greensville-Mecklenburg	*T. H. Valentine, M.D.	Lawrenceville	" " "
Dickenson-Lee-Scott-Wise	Dr. C. H. Reagan	Norton	" " "
Fairfax	*Adrian L. Carson, Jr., M.D.	Fairfax	" " "
Halifax-Pittsylvania	Dr. Daniel C. Steelsmith	South Boston	" " "
Henrico	*Dr. J. N. Dudley	Richmond	" " "
Isle of Wight-Nansemond-Suffolk	**William F. Wild, M.D.	Suffolk	Director of Health Dept.
Montgomery	Dr. J. B. Porterfield	Christiansburg	County health officer
Norfolk-Princess Anne	*J. Leake, M.D.	Portsmouth	" " "
Northampton	Dr. Albert B. McCreary	Eastville	" " "
Nottoway-Prince Edward	*W. A. Brumfield, M.D.	Farmville	" " "
Peninsula Health District	Dr. George E. Waters	Williamsburg	" " "
Rockbridge-Alleghany	*Robert P. Cooke, M.D.	Lexington	" " "
Southampton	*P. P. Causey, M.D.	Courtland	" " "
Valley District	Dr. S. D. Gardner	Harrisonburg	" " "
Wythe	Dr. David H. Andrew	Wythevill.	" " "
Washington			
Chelan	*C. R. Fargher, M.D.	Wenatchee	County health officer
Clark	Dr. Clyde B. Hutt	Vancouver	Health officer
King	*Wallace D. Hunt, M.D.	Seattle	County health officer
Spokane	*Dr. A. R. Lien	Spokane	" " "
Walla Walla	**Jerry E. Vanderpool, M.D.	Walla Walla	Health officer
Yakima	**Lloyd Moffitt, M.D.	Yakima	County health officer
West Virginia			
Berkeley	*C. A. Thomas, M.D.	Martinsburg	Health commissioner
Boone	*R. L. Hunter, M.D.	Madison	County health officer
Fayette	*H. H. Puckett, M.D.	Fayetteville	" " "
Hancock	*T. E. Cato, M.D.	New Cumberland	" " "
Harrison	*A. J. Kemper, M.D.	Clarksburg	" " "
Kanawha	**John Thames, M.D.	Charleston	" " "
Logan	T. J. Farley, M.D.	Logan	" " "
Marshall	*W. G. C. Hill, M.D.	Moundsville	" " "
Monongalia	*R. C. Farrier, M.D.	Morgantown	" " "
Ohio	*Reece M. Pedicord, M.D.	Wheeling	City & county health commissioner
Preston	*C. Y. Moser, M.D.	Kingwood	County health officer
Raleigh	*W. W. Hume, M.D.	Beckley	Health officer
Wood	**Arthur D. Knott, M.D.	Parkersburg	County health commissioner

* Member, A.P.H.A. ** Fellow A.P.H.A.